Does anthropometric and fitness parameters mediate the effect of exercise on the HRQoL of overweight and obese children/adolescents?

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Abstract

Background

There is sufficient evidence about the effects of physical exercise programs on health-related quality of life (HRQoL) in obese and overweight children.

Objectives

The purpose of this study was to observe the effects on physical fitness and HRQoL in overweight and obesity children and their parents and find out whether the effect of intervention on anthropometric and physical fitness parameters mediated the improvements found in the proxies' perception of participant quality of life.

Methods

151 overweight and obese children (106 intervention and 45 control) participated in a public exercise program. Anthropometrics characteristics, physical fitness, and HRQoL (EQ-5D-Y) were measured. Analysis of Covariance and effect size were performed to analyze the improvement. Mediation analyzed with bootstrap to observe whether anthropometric or physical fitness improvements mediate of the changes in the proxies' assessment of HRQoL.

Results

Significant improvements were found in waist circumference, physical fitness, and HRQoL. The improvement of waist circumference showed a significant indirect effect on the change in the proxy perception of quality of life.

Conclusion

The reduction of waist circumference mediates the change on proxies' perception of quality of life and not by the improvement in physical fitness. Trial registration: ISRCTN97887613

Background

It has already been extensively documented that overweight and obesity in children and adolescents entails physical and psychological comorbidities such as hypertension, hyperinsulinemia, dyslipidemia, type 2 diabetes, anxiety, and depression [1]. Thus, obesity and overweight in childhood are preceded by lower physical activity (PA) and higher sedentary behavior, causing detrimental effects on health-related quality of life (HRQoL) [2]. Several studies have documented significant improvements in HRQoL through programs based on physical exercise and sports practice in overweight and obese children and adolescents [2].

On the other hand, the assessment of the HRQoL of the participants in these programs can be made via self-report. However, when the children cannot answer the questionnaire themselves due to illness or inability to read or understand the instrument, there are proxy versions of some scales widely used today. The proxy assessment provides an additional perspective with the goal of complementing the evaluation of HRQoL; in no case should it replace the self-report assessment [3, 4].

Several longitudinal studies have been carried out in overweight and obese children to know the effect of some therapies on HRQoL [5] and in programs based on physical exercise [6], evaluated via self-report and proxy report, which show significant improvement with both instrument versions. Nevertheless, these studies did not mention which factors influence the proxy-parents' perception of the children's HRQoL in spite of there being moderate agreement between self-report and proxy report versions [2]. Mediation analysis is a statistical procedure which allows researchers to know the relationship between two variables and the degree to which this relationship can be mediated or confused by a third variable [7]. For example, it can be used to know which variables mediate the effect of exercise intervention in the proxies' HRQoL perception in overweight and obese children/adolescents. The purpose of this study was to analyze the effect on the proxy-assessed HRQoL for overweight/obese children after participation in exercise intervention and to analyze whether this intervention's effect on anthropometric and physical fitness parameters mediated the changes found in the proxies' perception of participant quality of life. Additionally, effects on HRQoL in self-report and self-report/proxy agreement were tested.

Materials and methods

A quasi-experimental study was carried out in overweight and obese children enrolled in a physical exercise program over 6 months, between January and June 2008, to discover the effects on anthropometrics and HRQoL compared to a control group. The study was supported by the University of Extremadura, Spain.

Participants and design

The study subjects consisted of 151 overweight and obese children (71 girls, 47%) who were assessed in the framework of a real public service supported by regional government

funds and was carried out by the University of Extremadura, Spain. The intervention group consisted of referrals to an exercise program detailed below, while the control group continued with their normal life after being evaluated at the same time as the experimental group. The intervention program was carried out in three different cities in the region of Extremadura (Spain), and the exercise program, as well as the evaluations, was directed by a graduate in Sports Science with experience in the use of the test battery and questionnaires explained below.

The sample was recruited with an advertising campaign by primary care centers, schools, sports clubs, and community neighborhoods promoting participation in the study through an information stand where posters and flyers were distributed to parents, teachers, social workers, nurses, and physicians. The campaign included the following: (a) support of the study from the regional government and the university, (b) participants did not pay any fees, (c) participants received an individual health-related fitness report after taking part in the test battery and completing the HRQoL questionnaire, (d) participants would undergo a short medical examination to ensure that they could perform the PA safely.

The participants had to comply with the following inclusion criteria: (i) be overweight or obese; (ii) have the ability to move themselves; (iii) not suffer from any disease that made it impossible to do PA; (iv) sign the informed consent themselves or have their parents do so; (v) understand each item from the questionnaire and (vi) perform the test battery safely.

The research has international registered (ISRCTN97887613), was approved by the Committee on Biomedical Ethics of the University of Extremadura (Ref. 98/2007), and followed the precepts of the Declaration of Helsinki.

Physical exercise program

The program offered 2 h/week of supervised PA divided into two sessions a week over 6 months, 60 min per day. The program was more focused on empowering children to gain resources to play with others than sport or short-term physical fitness performance. Activities consisted of physical activities in fun group dynamics to teach basic technical sport skills (soccer, basketball, athletics—running and jumping—walking with stilts, hockey without skates), social skills (forming groups or managing to play during playground or leisure time) and basic multiple physical fitness (velocity, strength, range of motion, coordination, and agility). The intended target was to prepare kids to partake in physical activities with other children and avoid possible bullying due to their minimal previous conditioning or performance.

Procedure and measurements

A set of questionnaires including demographic questions, weekly level of PA and HRQoL was administered as well as a battery of fitness tests.

The participants were instructed to wear sportswear and sneakers and not eat heavy food 2 h before as well as not to perform vigorous PA 48 h before the assessment. Before starting

the fitness test battery, the children performed an 8-min warm-up including running and joint mobility exercises. The questionnaires for children were completed by an experienced researcher in the subject on the first day before starting the program and again on the last day. The proxy-parent assessment was completed at the beginning and end of the program in meetings with them.

Anthropometric data

Weight and height: They were assessed with the participants standing barefoot in minimal clothing. The instrument used was a Seca 769 (Seca, Hamburg, Germany) scale with a transportable Seca 220 (Seca, Hamburg, Germany) stadiometer, which was placed on a rigid wall, with an accuracy of 0.1 cm. BMI was calculated as body weight divided by height squared (kg/m^2). The BMI variable was categorized into overweight and obese, according to indications by Cole et al. [8].

Waist circumference: It was measured at the level of the iliac crest with a non-elastic tape measure in meters by trained health measurement specialists. Reference values for Spanish children/adolescents could be consulted in the study of Moreno et al.

Health-related physical fitness

Cardiorespiratory fitness (CRF): the 20-m shuttle run test was performed to assess aerobic endurance. Each participant completed only one trial of this test that consisted of running back and forth between two markers spaced 20 m apart at a specified minimum pace. Successive audible beeps indicated the pace. The initial speed was 8.5 km/h and increased by 0.5 km/h every minute. Therefore, the number of shuttles run by each subject corresponded to the maximum velocity achieved. The test ended when the subjects failed to reach the finish line by the beep signal on two consecutive occasions. The total distance covered was recorded as the final result of the test [9].

Upper limb strength: It was measured using a handgrip dynamometer (TKK 5401 model, Tokyo, Japan) following the standardized protocol [9]. Two measurements were taken for each hand and the sum of the best for each hand was recorded.

Central body strength: It was assessed using the curl up test. This test consists of performing the maximum number of repetitions of sit-ups (up and back) in 30 s. Only one trial for this test was done.

Lower body strength: It was assessed using the countermovement vertical jump (CMJ) and standing long jump (SLJ). The CMJ was conducted using a contact platform connected to an electronic timer (Chronojump Bosco system, Barcelona, Spain). The CMJ was done with the subjects starting in a standing position with feet shoulder-width apart and hands placed on the pelvic girth [10]. The SLJ was done with the subjects standing behind the line and jumping as far forward as possible. The distance was measured in m from the starting line to the heel of the foot nearest the starting line [9].

Range of movement (ROM): the sit and reach test was done to assess ROM in pelvic and lumbar bending, recording the measurements in cm and using the best of two attempts in the statistical analysis [11].

Agility: a 4 × 10-m shuttle run test was performed to assess agility and speed. The subjects ran between two parallel lines marked 10 m apart on the floor as fast as possible, crossing each line with both feet every time. The stopwatch was stopped when the subject crossed the line for the fourth time. Two trials were done and the best result was recorded [9].

Health-related quality of life

EQ-5D-Y questionnaire and its proxy version: the Spanish version of the EQ-5D-Y (25) questionnaire and its proxy version [12] include a descriptive section made up of five domains with three answer levels ("no problems," "some problems," and "a lot of problems"). The questionnaire domains are mobility, self-care, usual activities, pain and discomfort, and anxiety and depression. It also includes a visual analogue scale (VAS) for the subject to perform an overall assessment of his/her self-perceived health status compared to the best imaginable health status that child could imagine on a visual scale from 0 to 100, where 0 were labeled as the worst health status and 100, the best health status child could imagine. Therefore, the child marked the percentage of health status compared with the best imaginable.

The proxy version, which is completed by parents (father or mother) in relation to their child's HRQoL, has the same characteristics of the self-report version.

Statistical analysis

The descriptive statistics are presented as mean (*M*) and standard deviation (SD). Before the analysis, the normality of data distribution was checked using the Kolmogorov–Smirnov test.

Changes from baseline to post-intervention in BMI, waist circumference, fitness tests, and VAS were analyzed using the Analysis of Covariance (ANCOVA) with adjustment for baseline values, age, and gender and the Chi-squared for EQ-5D-Y dimensions. Effect size (ES) was calculated using Cohen's *d* [13]. The level of agreement in the responses to the five domains of the EQ-5D-Y questionnaire and its proxy version was analyzed using Cohen's kappa coefficient in accordance with Landis and Koch's criteria [14], and the following levels of agreement were established: null: < 0; poor: 0–0.20; weak: 0.21–0.40; moderate: 0.41–0.60; good: 0.61–0.80; and very good: 0.81–1.00, together with an intraclass correlation coefficient (ICC) for the VAS. ICC values corresponded to null agreement: < 0.20; poor agreement: 0.21–0.40; fair agreement: 0.41–0.59; good agreement: 0.60–0.74; and excellent agreement: 0.75–1.0.

The *z*-scores adjusted by age and gender for fitness tests were calculated obtaining a variable for each test except the SLJ and CMJ which were combined (employing *z*-scores in these tests) as one variable called "lower limb strength." To obtain a global fitness index, a

new variable was calculated using the mean values of all *z*-scores (except the SLJ and CMJ which were included using the previously calculated lower limb strength variable).

Parallel mediation analysis was conducted to test whether the effect of the exercise intervention on fitness, computed as *z*-score, and whether waist circumference mediated the changes obtained in the proxies' perception of participants' quality of life (VAS). The mediation analysis was performed using data from all participants previously grouped in a dummy variable (0 = control/1 = exercise). Baseline values, age, and gender were used as covariates following Hayes' recommendations. The mediation analysis was done using the PROCESS macro for SPSS, version 2.16, developed by Hayes [15] with a bootstrap threshold of 10,000. If zero was not included in the 95% confidence interval (CI) of the estimate, we concluded that the indirect effect (IE) was statistically significant.

Results

One hundred and fifty-one overweight or obese children and adolescents and their parents participated in the study. The total sample was divided into two groups. The control group consisted of 45 subjects (53% female), ranging in age from 6 to 13 (*M* 8.7, SD = 1.6) and the experimental group included 106 participants (55% male), ranging in age from 6 to 14 (*M* 9.6, SD = 2.1). The participants did not suffer from other comorbidities as diabetes type I, hyperactivity, morbid obesity or contraindications to do exercise (biological or mental). Group BMI, waist circumference, and fitness characteristics are found in Table 1. There were not significant differences from baseline to post-exercise program for BMI, but there were for waist circumference. Additionally, there were significant differences in all fitness components except for CMJ and upper limb strength. The ES of these improvements were small for fitness components and moderate for waist circumference.

Table 1 Mean ± SD and standardized Cohen'*d* differences between baseline and 6-month post-intervention in anthropometrics measures and fitness components in exercise group and control group

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	Control group ($n = 45$)		Exercise group ($n = 106$)		*p* Value*	Cohen's *d*
	Pre	Post	Pre	Post		
BMI	25.02 ± 3.05	25.49 ± 3.34	25.53 ± 3.84	25.36 ± 3.90	0.12	0.17
Waist perimeter (cm)	80.58 ± 8.03	83.09 ± 7.90	84.01 ± 11.78	81.03 ± 12.21	0.00	0.52
CRF (m)	187.44 ± 136.95	245.16 ± 137.64	226.60 ± 179.14	292.08 ± 161.43	0.02	0.25
Upper limb strength (kg/cm^2)	26.59 ± 6.84	30.68 ± 8.34	38.96 ± 10.97	41.29 ± 12.94	0.21	− 0.27
CORE strength (n° × rep)	17.11 ± 11.93	20.67 ± 12.35	22.12 ± 18.09	28.88 ± 19.64	0.00	0.39
Lower limb strength						
CMJ (cm)	18.31 ± 3.44	19.69 ± 4.99	21.46 ± 4.65	23.92 ± 5.83	0.06	0.20
SL jump (cm)	92.15 ± 29.61	101.18 ± 19.26	107.62 ± 21.93	111.68 ± 21.54	0.00	0.37
ROM (cm)	22.33 ± 7.19	22.98 ± 5.53	19.89 ± 8.71	22.06 ± 7.42	0.00	0.22
Agility (sec)	15.40 ± 1.05	14.52 ± 1.80	14.66 ± 1.75	14.10 ± 1.62	0.00	0.24

Data are mean ± SD

Cohen's *d* adjusted by pooled standard deviation with weights for the sample size

**P* value = statistical significance computed by ANCOVA, adjusted by gender, age and baseline values as covariates

Regarding effects on HRQoL (Table 2), the ANCOVA revealed statistically significant differences in VAS between baseline and post-exercise program measurements in both the self-report and proxy versions. The ES for these changes were moderate for both self-reported and parent-proxy assessment. Thus, there were significant differences in pre- and post-intervention results for all EQ-5D-Y dimensions except in mobility dimension for both self-report and proxy.

Table 2 Mean ± SD, frequency (%), and significant differences between baseline and 6-month post-intervention in HRQoL PROs in exercise group and control group

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Dimensions	Children				*p*	*ES*	Parents				*p*	*ES*
	Control group ($n = 45$)		Exercise group ($n = 106$)				Control group ($n = 45$)		Exercise group ($n = 106$)			
	Baseline	Post	Baseline	Post			Baseline	Post	Baseline	Post		
EQ-5D-Y mobility					.08		±	±	±	±	.08	
No problems	48 (98.0)	40 (81.6)	110 (90.9)	118 (97.5)			46 (93.9)	41 (83.7)	107 (88.4)	114 (94.2)		
Some problems	1 (2.0)	9 (18.4)	11 (9.1)	3 (2.5)			1 (2.0)	7 (14.3)	4 (11.6)	7 (3.3)		
Many problems	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)		
EQ-5D-Y LAM*					.00		±	±	±	±	.01	
No problems	47 (95.9)	37 (75.5)	112 (92.6)	116 (95.9)			47 (95.9)	44 (89.8)	114 (94.2)	117 (96.7)		
Some problems	2 (4.1)	12 (24.5)	8 (6.6)	5 (4.1)			1 (2.0)	4 (8.2)	7 (5.8)	4 (3.3)		
Many problems	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)			1 (2.0)	1 (2.0)	0 (0.0)	0 (0.0)		
EQ-5D-Y UA*					.00		±	±	±	±	.00	
No problems	44 (89.8)	38 (77.6)	108 (89.3)	117 (96.7)			48 (98.0)	42 (85.7)	109 (90.1)	118 (97.5)		
Some problems	5 (10.2)	11 (22.4)	13 (10.7)	4 (3.3)			1 (2.0)	7 (14.3)	11 (9.1)	3 (2.5)		
Many problems	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)		
EQ-5D-Y P/D*					.00		±	±	±	±	.00	
No problems	34 (69.4)	39 (79.6)	98 (81.0)	117 (96.7)			35 (71.4)	36 (73.5)	98 (81.0)	112 (92.6)		
Some problems	15 (30.6)	10 (20.4)	23 (19.0)	4 (3.3)			12 (24.5)	13 (26.5)	23 (19.0)	8 (6.6)		
Many problems	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			2 (4.1)	0 (0.0)	0 (0.0)	1 (0.8)		
EQ-5D-Y WSU*					.00		±	±	±	±	.00	
No problems	45 (91.8)	36 (73.5)	105 (86.8)	119 (98.3)			43 (87.8)	33 (67.3)	104 (86.0)	111 (91.7)		
Some problems	4 (8.2)	13 (26.5)	15 (12.4)	2 (1.7)			5 (10.2)	13 (30.6)	15 (12.4)	10 (8.3)		
Many problems	0 (0.0)	0 (0.0)	1 (0.8)	0 (0.0)			1 (2.0)	1 (2.0)	2 (1.7)	0 (0.0)		
EQ-5D-Y VAS	84.7 ± 16.3	86.1 ± 15.8	81.6 ± 16.6	86.1 ± 15.5	.00	0.78	90.3 ± 11.5	85.3 ± 13.6	84.7 ± 12.7	90.1 ± 10.0	.00	0.79

Data are mean ± SD. Cohen's *d* adjusted by pooled standard deviation with weights for the sample size

**P* value = statistical significance computed by ANCOVA, adjusted by gender, age, and baseline values as covariates

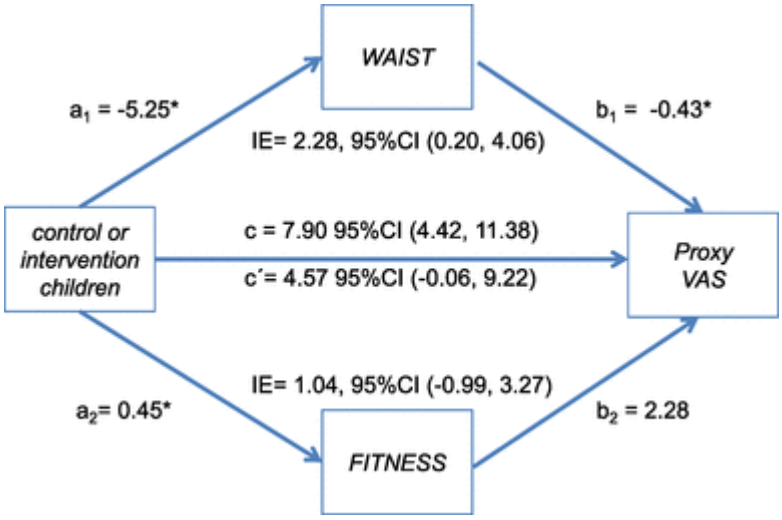
*LAM (Looking After Myself), *UA (Usual Activities), *P/D (Pain/Discomfort), *WSU (Worried, Sad or Unhappy)

The agreement analysis between self-report and proxy versions are shown in supplementary Table. This analysis showed moderate agreement for the "mobility" and "worried, sad or unhappy" dimensions at baseline while being poor for all the other EQ-5D-Y dimensions and VAS. However, after 6 months of intervention, child/parent agreement was good for VAS; fair for the "pain/discomfort" and "worried, sad or unhappy" dimensions; and poor for the "mobility," "looking after myself," and "usual activities" dimensions.

Finally, Fig. 1 shows results of the parallel mediation analysis to know whether the effect of this intervention on fitness and waist circumference mediated the changes found in the proxies' perception of participants' quality of life (VAS). The exercise program was positively associated with an increase in parents' perception of their child's HRQoL when the improvements in fitness and waist circumference were included in the model [total effect (*c*): *IE* = 7.90; 95% CI 4.42, 11.38]. However, the mere fact of participating in the program did not have any significant direct effect (*c*′) on the parents' perception of their child's HRQoL (*IE* = 4.57; − 0.06, 9.22). Finally, a mediator effect was observed for waist circumference regarding the changes obtained in the proxies' perception of participants' quality of life (VAS) (*IE* = 2.28, 95% CI 0.20, 4.06). However, the indirect effect on physical

fitness was not significant, and therefore, it was not a mediator in this relationship (*IE* = 1.04, 95% CI − 0.99, 3.27).

Fig. 1



Paralell mediation analysis of intervention condition on health self-perception (proxy VAS) through waist circunference or physical fitness, adjusted by baseline scores. Number of bootstrap samples = 10,000. The indirect effect is statistically significant at the 95% confidence interval (CI) when the CI does not include 0

Full size image

Discussion

To the best of our knowledge, no previous studies have examined the mediation role of the improvement in anthropometric and physical fitness parameters after exercise intervention for overweight and obese children on their parents' perception of their HRQoL. The main findings in this study indicate that the reduction in waist circumference mediates the effect of the exercise intervention in the parents' perception of the participant's HRQoL, while improvements in physical fitness parameters did not. These results suggest that physical exercise should focus on reducing abdominal obesity to improve parents' perception of overweight/obese children's HRQoL. These findings help to understand the evaluation of HRQoL in this population using the parent's perspective through proxy instruments.

Literature examining the variables which mediate the parent's perception of children's HRQoL is scarce. There are some previous studies analyzing the reason for the disagreement in the evaluation of HRQoL between parents' and overweight or obese children's perspectives through causal relationship, but none through the analysis of indirect effects. Tsiros et al. [2] found several variables in a review that may influence this disagreement such as the parents' concern about the consequences of weight gain on physical and psychosocial functioning. Our study found that the body image that parents have of their children mediates their perception of the children's health, which is related to

weight gain or loss. However, the effects of exercise on physical performance, which can affect the activities of daily living, did not influence changes in the perception that proxies have about children's health. This may be due to the emotional impact that the children's nutritional status has on the parents and the parents' concern about the consequences of weight gain in future life, as documented in previous studies [16, 17]. Therefore, the improvement in body volume thanks to children's participation in physical exercise programs influences the perception that parents have, mediating this improvement in the perception of HRQoL. Literature about mediation analysis in this topic is limited, and performing a simple comparison would be incorrect.

Regarding the effect of the physical exercise program on physical fitness, there were significant improvements in CRF, core, SLJ, ROM, and agility, but with a small ES. Nonetheless, there were non-significant effects on upper limb strength and CMJ. A moderate ES improvement was also obtained in waist circumference. These results are consistent with previous studies [18, 19] whose effects are similar, although such studies are based on programs which aimed to improve fitness level. In our study, the aim was improving the social skills to empower children in physical exercise. The number of hours in these studies was also higher than in our study.

Additionally, the exercise intervention showed a significant improvement in VAS and EQ-5D-Y dimensions, except in mobility in both the self-report and proxy instruments. These findings are similar to previous studies done with obese or overweight children [20, 21]. However, the lack of improvement in the mobility dimension may be due to a ceiling effect presented by this questionnaire [22].

This study also examined the agreement between the evaluation of children's HRQoL assessed by EQ-5D-Y and its proxy version both at baseline and post-intervention. Such agreement was poor, fair or moderate. Parents slightly overestimate perceived improvement in their children's HRQoL in the exercise group and somewhat underestimate it in control group. Therefore, parents perceive their child's participation in the exercise program as positive for HRQoL. In this sense, information provided directly by children, especially in longitudinal studies, may be less consistent due to the changes in attitudes, skills, and priorities characteristic of child development [2]. Nonetheless, the perception of HRQoL by proxy may be influenced by different factors, e.g., intrinsic factors like parental stress [23], a catastrophic point of view about the effect of weight status on functioning or limited understanding about their child's experience. Therefore, the results found were also similar to several studies [2, 24].

Strengths

The present study has shown a novel analysis perspective to find out whether the effects produced in anthropometric measurements and physical fitness level in overweight and obese children after their participation in an exercise program mediate changes in the proxies' perception of participants' HRQoL. Therefore, mediation analysis helps us to understand which variable influences the parent-proxy perception. As a result, our findings

provide a first step to understand the agreement–disagreement between self-report and parent-proxy report. Furthermore, mediation analysis could be an additional tool to clarify the lack of evidence in this field of research. As we can see in the mediation figures, the direct effect is not significant. In other words, the zero value crosses the confidence interval. It is possible that there is an indirect effect without a statistically significant direct and total effect. This would explain that the X and Y are not linearly related, since the correlation is neither necessary nor sufficient for causation [15] and the sample size also might not be strong enough to reach significance [7]. The statistical method employed to find disagreement may also influence agreement. The Pearson correlation is the most frequent method applied, but this only informs about the covariation among scores, it does not indicate absolute agreement [25]. In this case, the intraclass correlation coefficient (ICC) is more appropriate since it provides the ratio between subject variability and total variability [26].

Limitations

One of the main limitations of the study is the non-randomization and the sample size of the groups since they should be more balanced. However, this was corrected with 10,000 bootstrap samples. Another limitation was the program's exercise intensity. This can be observed in the non-significant effect on BMI and could be due to the fact that the program consisted of 2 h/week or as a result of BMI's inability to detect changes in children's weight status [27]. Nevertheless, our exercise program aimed to provide tools to empower them in PA and not to improve their physical fitness. However, there are previous studies with similar results based on virtual activity [28] or face-to-face PA [29]. Another limitation is the proxy responder, since our study did not take into account whether it was the mother or father who answered the questionnaire.

Conclusions

According to the results obtained in this study, parents' perception of HRQoL for overweight or obese children who participated in an exercise program was mediated by the reduction in waist circumference and not by the improvement in physical fitness. Therefore, the proxies' perception of participants' quality of life is influenced by the children's anthropometric changes. Thus, future exercise programs for overweight or obese children should focus on reducing abdominal obesity to improve the parents' perception of the overweight/obese child's HRQoL.

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Funding

The research has international registration (ISRCTN97887613) and was funded by University of Extremadura and Health & Dependence Department and Young & Sports Department of Junta de Extremadura. (Ref. 118/06) (Spain).

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Contributions

All authors participated in the research as well as the preparation of the manuscript.

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Ethics declarations

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The study was approved by the Bioethical Committee of the University of Extremadura on 31 October 2007 (Ref: 98/2007). All authors know and ensure that the ethical principles proposed in the Helsinki Declaration and subsequent reviews for human studies have been fulfilled and that they guarantee the privacy and confidentiality of patient data at all times.

Informed consent

The subjects agreed to the study terms signing an informed consent document.

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