Effects of exercise in addition to a family-based lifestyle intervention program on hepatic fat in children with overweight

Short running title: Additional effect of exercise on hepatic fat

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ABSTRACT

Background: Paediatric hepatic steatosis is highly prevalent and closely related to type 2 diabetes.

Aims: To determine whether the addition of supervised exercise to a family-based lifestyle- and psycho-educational intervention results in greater reduction of percentage hepatic fat (HF), adiposity, and cardiometabolic risk factors in children with overweight/obesity.

Methods: The study subjects of this non-randomized, two-arm, parallel design, clinical trial were 116 overweight/obese children (10.6±1.1 years, 53.4% girls) living in Vitoria-Gasteiz (Spain). For 22 weeks they followed either a lifestyle- and psycho-education program (control intervention [CI], N=57), consisting of two family-based education sessions/month, or the same plus supervised exercise (intensive intervention [II], N=59) focused mainly on high-intensity aerobic workouts (3 sessions/week, 90 min/session). The primary outcome was the change in percentage HF (as measured by MRI) between baseline and the end of the intervention period. Secondary outcomes included changes in body mass index (BMI), fat mass index (FMI), abdominal fat (measured by dual-X-ray-absorptiometry), blood pressure, triglycerides, high density lipoprotein, low density lipoprotein, gamma-glutammyl-transferase, glucose and insulin concentrations.

Results: A total of 102 children completed the trial (N=53 and N=49 in the CI and II groups, respectively). Percentage HF decreased only in the II group (-1.20±0.31% *vs.* 0.04±0.30%, II and CI, respectively), regardless of baseline value and any change in adiposity ($P<0.01$). BMI, FMI, abdominal fat ($P\leq0.001$) and insulin ($P<0.05$) were reduced in both groups.

Conclusions: Multicomponent intervention programs that include exercise training may help reduce, adiposity, insulin resistance and hepatic steatosis in overweight/obese children.

KEYWORDS: hepatic steatosis, childhood, physical activity, nutrition, psychology, obesity

ClinicalTrials.gov ID: NCT02258126

INTRODUCTION

Children with overweight/obesity are at greater risk of developing cardiovascular diseases (CVD), type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) in adulthood[1,2], and even in childhood.[3] Paediatric NAFLD is affecting to nearly 34% of children with obesity.[4] It is estimated that by 2025, 38 million will have hepatic steatosis, the earliest manifestation of NAFLD.[5]

Paediatric hepatic steatosis is an independent risk factor for type 2 diabetes.[6,7] Excessive body mass gains during childhood may eventually induce the histological features of adult NAFLD.[8] The treatment of overweight/obesity before puberty, however, can reduce the risk of developing type 2 diabetes in adulthood[9]. The early treatment of childhood overweight/obesity and its comorbidities is therefore vital.

Currently, there is no pharmacological options for NAFLD in children.[10] Lifestyle modification is therefore the primary option.[10] A systematic review of lifestyle interventions for the treatment of overweight/obesity in children aged 6-11 years observed that multicomponent behavior-changing programs achieved small, short-term reductions in body mass index (BMI).[11] However, no studies have examined the effect of a non-hypocaloric diet-based lifestyle intervention program on percentage hepatic fat (HF) in children.

Exercise training seems to be effective in reducing HF in adolescents[12], and in reducing the risk of developing CVD and type 2 diabetes[13] in children. In a systematic review,[12] it was reported that supervised exercise, designed following the international recommendations of physical activity, reduced HF in youths. However, there has been no such study exclusively involving children under 12 years of age, and certainly none that has employed imaging methods to assess HF.

Given the above, lifestyle and behavioral modifications plus exercise training might be expected to have greater effects on HF (as well as on children's health and psychological well-being) than interventions based on lifestyle modifications alone. The aim of the present work was to determine whether a multicomponent intervention program designed according to current evidence and guidelines, and including a family-based lifestyle and psycho-education program plus a supervised exercise intervention, is more effective at reducing percentage HF than the lifestyle program alone, in children with overweight/obesity aged 8 to 12 years. The effects of these interventions on fat mass, CVD and type 2 diabetes risk factors, physical fitness, dietary habits, physical activity and psychological well-being, are also discussed.

MATERIALS AND METHODS

Study design

The EFIGRO project is a non-randomized two-arms parallel-design controlled trial (ClinicalTrials.gov ID: NCT02258126).[14] The study was conducted at the University of the Basque Country in Vitoria-Gasteiz (northern Spain), from September 2014 to June 2017. The Euskadi Clinical Research Ethics Committee approved the study protocol (PI2014045), which complies with the ethical guidelines of the Declaration of Helsinki (2013 revision). All parents/legal guardians gave their informed, written consent for their children to be included in the study; the children also gave their assent before enrolment.

The EFIGRO project compared differences on changes in percentage hepatic fat, cardiometabolic and diabetes risk factors between two groups: one group received a family-based lifestyle and psycho-educational program (hereinafter referred as the control intervention, CI) and another group received the same intervention plus an exercise program (hereinafter referred as the intensive intervention, II).

Participants

Children and their families were recruited at the Pediatric Endocrinology Unit of the University Hospital of Araba, and at Primary Care Clinics in the city of Vitoria-Gasteiz. The main entry criterion was having overweight/obesity, as defined by the cut-offs for sex and age (by month) established by the World Obesity Federation.[15] All children had to be aged 8-12 years. Subjects whose medical condition or medication limited their physical activity, or that might affect the results obtained, were excluded. None of the children had diabetes mellitus or any other endocrine disorder, and all were non-smokers.

Assignment to intervention arms and blinding

Children were allocated into the CI or the II groups after baseline measurements. The design of the current project was conceived as a RCT; however, this assignment was not completely random. A number of children/ families (N=11) did not have time to attend the exercise sessions and were thus allocated to the CI group. This decision was taken since the children/families had been encouraged to participate in the study by their pediatricians, citing probable health benefits.

Neither the staff delivering the intervention nor the study subjects were blinded to the arm assignment. However, the researcher in charge of analyzing the outcomes were thus blinded.

Intervention protocols

Lifestyle and psycho-educational program: Both the CI and the II groups, participated in the lifestyle and psycho-educational program. Parents/caregivers and children attended separately the lifestyle (45 min) and psycho-educational (45 min) program once every two weeks (i.e., 11 sessions in total), as explained in detail elsewhere.[14] The focus of the program was to increase 1) the parents' and children's knowledge about healthier dietary habits, 2) their physical activity level, and 3) to promote sleep hygiene. The aim of the the psycho-educational program was to provide skills to the parents/caregivers in order to optimize the family environment for making positive lifestyle changes, and to learn assertive communication skills. The program also provided skills to children for managing their emotions and feelings, and improving their self-esteem and psychological well-being.

Design of the supervised exercise program: Only the II group subjects took part in the exercise program. The full design of the program is available elsewhere.[14] Children attended three sessions per week. Briefly, sessions were designed and supervised by exercise specialists and consisted of 5 min instruction time, 10 min of warm-up, 60 min of game-based cardiovascular endurance, 10 min of muscle strength exercises, and 5 min of cooling-down and stretching exercises (overall, 90 min/session). Subjects were encouraged through motivation and game strategies to spend as much time as possible during the session in the vigorous-high intensity physical activity.[16] The maximum heart rate was defined as the highest value obtained during the cardiopulmonary exercise test in the laboratory, or in the 20msrt. Children wore a Polar RS300X HR monitor during the sessions for recording exercise intensity.[16]

Participant retention and addressing compliance and adherence: The attendance of both the children and their parents/caregivers at the lifestyle and psycho-educational sessions was recorded. In the exercise program, children were marked “absent” if they did not attend a session or refused to participate in the proposed games or activities. Regardless of their adherence record, all subjects were encouraged to return for post-intervention data collection

Measurements

Primary and secondary outcomes were assessed at baseline and after 22 weeks of intervention by the same trained researchers.

Primary outcome measure: percentage hepatic fat

Percentage HF was measured by magnetic resonance imaging (MRI) using a MAGNETOM Avanto 1.5T system (Siemens Healthcare, Erlangen, Germany) equipped with a phased-array surface coil and a spine array coil and and running Siemens Medical System software v.syngo.MR B17A, following the manufacturer's instructions.[17] Children with ≥5.5% HF were deemed to have hepatic steatosis.[18]

Secondary outcomes measurements

Physical examination and body composition: Body mass and height were measured in duplicate. The cut points established by the World Obesity Federation (http://www.worldobesity.org/) by sex and for each month of age were used to define overweight and obesity. [15] Pubertal stage was recorded by a pediatrician.[19] Systolic and diastolic blood pressure was measured following recommendations for children[20]. Participants were deemed hypertensive when their systolic or diastolic blood pressure was >90th age-, sex- and height specific percentile.[20]

Total and abdominal fat, as well as lean mass, were measured by dual energy X-ray absorptiometry using a HOLOGIC, QDR 4500W device. The fat mass index (FMI) was then determined as *[FMI: fat mass (kg)/stature^2 (m^2)] and lean mass index [LMI: lean mass (kg)/stature^2 (m^2)]*.

Physical fitness: Cardiorespiratory fitness (CRF) and muscle strength were determined following validated protocols for children.[21] Briefly, CRF was assessed by two tests: 1) an incremental CRF treadmill protocol with respiratory gas analysis until exhaustion, and 2) the 20m shuttle run test (20msrt). Upper and lower body muscular strength were assessed by the handgrip and the standing broad jump tests respectively.

Biochemical measurements: Plasma triglycerides (TG), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), insulin, glucose and gamma-glutamyl transferase (GGT) concentrations were determined as reported elsewhere.[14] The insulin resistance-homeostatic model assessment index (HOMA) and the TG/HDLc ratio were then calculated. Insulin resistance was defined according to the age- and sex-specific cut-points for HOMA values.[22] Subjects with TG/HDLc values of ≥2.0 were deemed to be at increased cardiometabolic risk.[23]

Physical activity, sedentary time, sleep and dietary habits assessment: Physical activity was determined by accelerometry (wGT3X-BT, Actigraph, Pensacola, FL, USA) and dietary habits were assessed using two non-consecutive 24h recall records and food frequency questionnaires, as detailed elsewhere.[14]

Psychological assessment: Anxiety was assessed using the State-Trait Anxiety Inventory for Children; stress was determined using the Children's Daily Stressors Inventory; self-concept was assessed using the Self-Concept Form-5 Questionnaire; and depression examined using the translated version of the Children's Depression Inventory.[14]

Statistical analyses

Since no information on the effect of exercise on percentage HF in children was available, the required sample size was calculated using information available for closely associated secondary outcome variables. The sample size actually obtained was N=116. This figure was expected to provide at least 80% power for detecting an effect-size (Cohen' d) between the two experimental groups of 0.7 for insulin resistance and total body fat (minimum number required in each group = 34 for 80% power and α=0.05).

The baseline characteristics of the subjects' in the two intervention groups were compared using either the Student-t-test (for continuous variables) or the Chi-squared test (for categorical variables). Differences between the intervention groups in terms of post-intervention values for the primary and secondary outcomes were also examined using the Student-t-test. Within-group differences (pre *vs*. post values) in primary and secondary outcomes were examined using the Student paired t test following per protocol and intention-to-treat principles. For the intention-to-treat analyses, missing values at follow-up were obtained by multiple imputation using the following predictor variables: pre-intervention values, post-intervention values, age, sex, and intervention group. Imputations were performed as four blocks: body composition variables (HF, body mass, BMI, FMI, LMI, abdominal fat), blood pressure variables (systolic and diastolic blood pressure), cardiometabolic risk variables (TG, HDLc, LDLc, TG/HDLc, insulin, glucose, HOMA, GGT), and fitness variables (VO_2 peak, endtime treadmill test, 20msrt result, handgrip strength, and standing broad jump).

In both the per protocol and the intention-to-treat analyses, differences between the CI and II groups in terms of changes in primary and secondary outcomes were examined by analysis of covariance adjusting for baseline values. Cohen's d was used to estimate the effect size and 95% confidence interval (95% CI). Differences between the CI and II groups in terms of changes in the percentage HF were examined in extended models: Model 1, unadjusted; Model 2, adjusted by the corresponding baseline value and changes in height; Model 3, adjusted by baseline values and changes in the FMI; Model 4, adjusted by baseline values and changes in abdominal fat.

Finally, the relationship between the changes (pre- to post-intervention values) in physical fitness variables and in percentage HF in each intervention group (CI and II) were examined by regression analysis, 1) using an unadjusted model, and 2) adjusting for baseline physical fitness and percentage HF.

Significance was set at $p<0.05$. All calculations were made using the Statistical Package for Social Sciences v.24.0 for WINDOWS (SPSS Inc, Chicago, IL, USA).

RESULTS

Figure 1 shows the flow chart for the trial, plus subject inclusions and exclusions. **Table 1** shows the participants' baseline characteristics. Overall, there were no significant differences in the socioeconomic, sociodemographic, anthropometric or clinical characteristics between the CI and II groups.

A total of 102 of the original 116 subjects (87.9%; N=53 in the CI group and N= 49 in the II group) successfully completed the trial, attending at least 50% of the sessions. Their data were included in the per protocol analyses. The data for those children that discontinued the intervention (N=4 in the CI group and N=9 in the II group, or who did not attend at least 50% of the educational program sessions (N=1, in the II group), were also included in the intention-to-treat analyses.

Attendance, adverse events and main characteristics of the program

No significant difference was seen between the CI and II groups in terms of attendance at the lifestyle- and psycho-education program sessions, either for the parents/caregivers (86.4±12.9% *vs.* 80.6±15.3%; P=0.334) or the children (87.2±12.0% *vs.* 82.5±14.6%; P=0.496). The mean attendance rate for the II subjects with respect to the exercise program was 72.0±16.1% sessions. Exercise-related adverse events included knee and ankle pain (N=2); no adverse events were recorded for the lifestyle- and psycho-education program.

In the exercise program, the mean HR per session was 146±16 bpm. High intensity exercise was maintained for 49±23% of the time, and moderate intensity exercise for 32±15% of the time.

Effects of the intervention on primary outcome

Percentage hepatic fat

No significant difference in percentage HF was seen between the CI and II groups at baseline (**Table 2** and **Supplemental Table 1,** for the per protocol and intention-to-treat analyses, respectively). Although there were no significant differences in post-intervention values of percentage HF between the two groups (Tables 2 and Supplemental Table 1), only children in the intensive group reduced percentage HF after the intervention (Table 2 and Supplemental Table 1, for the *per protocol* and *intention-to-treat* analyses, respectively). The difference in the change in percentage HF between the two groups (per protocol analysis) was significant (P<0.02, **Figure 2 and Supplemental Figure 1**); these results persisted after adjustment for baseline values for percentage HF and changes in height (-1.20±0.31 *vs.* 0.04±0.30% for the II and CI group respectively; P=0.006), and when changes in FMI (-1.13±0.27 *vs.* -0.02±0.27% for the II and CI groups respectively; P=0.004) and abdominal fat (-1.19±0.26 *vs.* -0.03±0.28% for the II and CI groups; P=0.004) were adjusted for instead of changes in height (Supplemental Figure 1). Further adjustment for baseline VO_2peak (measured via the treadmill test) did not substantially alter the results (P<0.05). The intention-to-treat analyses for differences between groups in terms of the change in percentage HF returned similar results (**Supplemental Figure 2).**

Effects of the intervention on secondary outcomes

Adiposity and CVD and type 2 diabetes risk factors

No significant differences were seen between the CI and II groups in terms of any of the studied cardiometabolic or type 2 diabetes risk factors at baseline (Tables 2 and Supplemental Table 1). Both groups returned significantly reduced BMI, FMI and abdominal fat values after the intervention (P<0.01), while no significant changes were seen in LMI (Table 2 and Supplemental Table 1 for the per protocol and intention-to-treat analyses respectively. No significant differences were seen between the groups in terms of post-intervention in any adiposity estimate (Table 2 and Supplemental Table 1). The reduction in BMI was significantly greater in the II group ($P<0.01$), but no significant differences were seen between the groups in terms of the change in FMI or abdominal fat (Figure 2). These results did not substantially differ after adjusting for baseline values (**Supplemental Figure 2**). The intention-to-treat analyses returned similar results (Supplemental Figure 3).

Per protocol analysis showed the insulin concentration and HOMA value to be significantly smaller ($P<0.01$ and $P<0.05$ respectively) in the CI group after the intervention (Table 2). These reductions were not significant, however, in the intention-to-treat analysis ($P<0.09$, Supplemental Table 1). In the II group, the HDLc ($P<0.01$), LDLc ($P<0.001$), insulin ($P<0.05$) and GGT ($P<0.05$) concentrations all decreased significantly by the end of the intervention program (per protocol analysis). Diastolic blood pressure increased significantly in the II group, while no significant change was observed in the control group (Table 2). The intention-to-treat analysis returned similar results, but only the changes in TG ($P<0.05$), insulin ($P<0.001$) and GGT ($P<0.01$) remained significant (Supplemental Table 1).

No significant differences were seen in terms of post-intervention cardiometabolic and diabetes risk factors between the two groups (Tables 2 and Supplemental Table 1), except with respect to the LDLc level ($P<0.05$; both per protocol and intention to treat). However, greater reductions in LDLc were seen in the II group than in the CI group after the intervention; the per protocol and the intention-to-treat analyses returned similar results (Figure 2, and Supplemental Figures 2 and 3).

In the per protocol analysis, significant difference was seen between the groups in terms of the effect of the interventions on TG ($P<0.05$, Table 2), but this was attenuated in the intention-to-treat analyses ($P<0.07$, Supplemental Table 1). However, the differences in the change in TG were not consistent between the per protocol (Figure 2 and Supplemental Figure 2) and intention-to-treat analyses (Supplemental Figure 3). Similarly, analysis-inconsistent differences were seen between groups in terms of the effect of the interventions on GGT levels (Figure 2, and Supplemental Figures 2 and 3).

Physical fitness

At baseline, VO_2peak as measured by the treadmill test was lower in the II than in the CI group ($P<0.05$), but no significant differences were seen in the rest of the fitness variables (Table 2 and Supplemental Table 1). Significant increases were seen in both groups for all physical fitness variables after the intervention ($P\leq0.001$, Table 2 and Supplemental Table 1), but no significant differences were seen between them in terms of the changes in these values (Figure 2), even after adjustment for baseline values (Supplemental Figure 2). The intention-to-treat analysis returned similar results (Supplemental Figure 3).

The increase in CRF measured in the 20msrt was significantly associated with the reduction in percentage HF content in the II group subjects -at least in the per protocol analysis (**Supplemental Table 2**). This relationship was attenuated and became non-significant ($P<0.06$) in the intention to treat analysis. (**Supplemental Table 3**).

Effects of the intervention on lifestyle and psychological well-being factors

All comparisons were per protocol (**Supplemental Table 4**). No significant differences in dietary and physical activity variables were seen between the two groups at baseline. By the end of the intervention, both groups had significantly reduced their energy and fat intake and increased their intake of fruits and vegetables. No significant changes were seen, however, in the consumption of sugar and sugar-sweetened beverages. Neither MVPA nor sleep time changed after the intervention in either group, although sedentary time was significantly reduced in the II group ($P<0.05$). No significant differences in dietary or physical activity variables were seen between the two groups at the end of the intervention.

No significant differences were seen in psychological variables between the two groups at baseline (Supplemental Table 4). By the end of the intervention, the subjects of both groups had experienced a significant increase in terms of emotional self-concept ($P<0.05$). Improvements were also seen in physical self-concept, total depression, dysphoria and anxiety, although they were only significant in the CI group ($P<0.05$ and $P<0.09$, for the CI and II intervention groups, respectively). No significant differences in the improvement in psychological well-being were seen between the two groups at the end of the intervention.

DISCUSSION

The present results reveal the II subjects to have experienced a clinically important reduction (nearly 20%) in percentage HF; no such reduction was seen in CI group. Both the CI and II subjects experienced a reduction in total and abdominal fat and insulin resistance. The II subjects also enjoyed a significant reduction in LDLc. Given the importance of treating pediatric hepatic steatosis early -not only to prevent future liver damage but also type 2 diabetes- these findings should be taken into account in pediatric obesity management programs.

Family-based lifestyle education programs accompanied with psychological support are recommended for the treatment and prevention of paediatric obesity and related comorbidities.[25] However, the the present study shows that while the educational program followed was able to effectively reduce adiposity and insulin resistance, it was unable to reduce the percentage HF of the children. Hepatic fat was only reduced in those who participated in the supervised exercise program in addition to the lifestyle education program. These results agree with those of a study involving obese adolescents[26], in which a 4-month intervention focused on changing the quality of carbohydrate intake resulted in significant improvements in insulin sensitivity -but only a combined lifestyle, psycho-education and resistance exercise training program resulted in a reduction in percentage HF.

As far as we are aware, the present work is the first to report the additional effect of supervised exercise on HF as measured by MRI in children. Other studies have reported significant reductions in HF after aerobic or resistance exercise training interventions, but in adolescents.[13,27–29] In a recent systematic review and meta-analysis, it was reported that exercise training at moderate-to-vigorous or vigorous intensity, in sessions of at least 60 minutes, and a frequency ≥3 sessions per week are effective reducing HF content in youth.[12] The present findings are in agreement with these recommendations.

In adults and adolescents with overweight or obesity, lifestyle interventions including exercise -or not- have been reported to significantly reduce HF.[30,31] However, these programs were based on hypocaloric diets that caused large body mass losses.[31,32] In the present study -which involved pre-adolescent children- the lifestyle education program was not designed to achieve body weight loss in the short term, and certainly the CI subjects lost no body mass or lean mass.

A systematic review and meta-analysis comparing the effect of exercise training and a hypocaloric diet on visceral adiposity[33] reported the latter to result in a larger body mass loss, whereas exercise training tended to induce larger reductions in visceral adiposity. Interestingly, in the present work, the effects of exercise on percentage HF were independent of the change in total or abdominal fat, suggesting a direct metabolic effect of exercise training on HF metabolism.

Family-based, structured lifestyle modification programs combined with behavioral strategies for treating obesity have been associated with adiposity reductions in children.[34] In the present study, the children's dietary habits were improved with respect to the development of obesity after the 22-week intervention. Improvements were also seen in several components of self-concept, depression, stress and anxiety in children. These improvements in both dietary habits and psychological health are quite notable.

Physical activity was not increased at the end of the intervention in either the CI or II group; indeed, in the CI group there was even a trend towards a reduction in total physical activity. It should also be noted that several families declined to participate in the II arm given the relatively large number of sessions involved. A possible solution might be to reduce the number of session per week or the duration of each session. Future studies examining the effectiveness of less intensive exercise programs on percentage HF and cardiometabolic risk in overweight/obese children are warranted.

One of the strengths of this study is the use of MRI for measuring percentage HF. Moreover, the sample size was also larger, and the duration of the intervention program longer than in previous studies.[13,27,28] Of note is, however, that several families did not agree to participate in the intensive intervention due to the relatively elevated number of sessions which may comprise the feasibility of the intensive program. There are, however, some limitations that should be mentioned. The most important limitation of the study is its not entirely strict randomization [14]. This may limit the validity of the results. However, the participants in both groups were comparable at baseline, and adjustments for potential baseline differences between groups were made in analyses. Finally, the results were not adjusted for multiple comparisons.

Conclusion

A family-based, multicomponent intervention program including supervised exercise training and lifestyle- and psycho-education, designed following international recommendations for obesity prevention and health promotion in children, was shown to have the potential to reduce HF, total and abdominal fat, and insulin resistance, and to improve dietary habits and psychological well-being, in pre-adolescents children with overweight/obesity. These findings highlight the importance of promoting such programs as part of pediatric obesity treatment: improvements may be achieved not only in total and abdominal fat and insulin resistance, but also in hepatic steatosis, a metabolic abnormality that increases the risk for type 2 diabetes and cardiovascular disease.

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Author contribution to manuscript

IL designed the study, analyzed the data and drafted the manuscript. IL takes full responsibility for the integrity of the data analyses. LA, MM, EM, and MO collected the data and critically revised the manuscript. IL, JRR, VMV and FOB participated in the interpretation of the results. All the authors critically revised the manuscript for its intellectual content and approved the final version. Dr. Labayen is the guarantor of this work, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

The authors declare they have no conflicts of interest.

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FIGURE LEGENDS

Figure 1. Flow chart showing enrollment into the study groups. Control intervention group: children participating in the family-based lifestyle- and psycho-educational program; Intensive intervention group: children participating in the latter plus exercise training.

Figure 2. Unadjusted effect size (d-Cohen and 95% CI) and differences in changes (Δ) in percentage hepatic fat, body mass and composition, cardiometabolic and diabetes risk factors between the control group (children participating in the family based lifestyle and psycho-educational program) and intensive groups (children participating in the multicomponent intervention including lifestyle and psycho-educational program). Differences between the control and the intensive intervention groups in terms of changes in primary and secondary outcomes were examined (per protocol) by ANOVA. Changes were calculated as post-intervention minus pre-intervention values. Data are means (standard deviation). *Negative Cohen's d values obtained for the differences in the changes in adiposity and cardiometabolic and diabetes risk between the two groups (in favor of the II group) were multiplied by -1 for illustrative purposes. HDL: high density lipoprotein cholesterol; HOMA: homeostasis model assessment; TG/HDL: triglycerides to high density lipoprotein ratio; LDL: low density lipoprotein; GGT: gamma glutamyl transferase; VO_{2peak}: peak oxygen consumption from the treadmill test; 20msrt: performance in the 20 meters shuttle run test.

Supplemental Figure 1. Change in percentage hepatic fat in the group of children participating in the lifestyle- and psycho-educational program (grey bars, control), and in those participating in the latter plus exercise training (white bars, intensive). Model 1:

unadjusted; Model 2: analyses were adjusted with baseline values and changes in height; Model 3: analyses were adjusted with baseline values and changes in fat mass index; Model 4: analyses were adjusted with baseline values and changes in abdominal fat. **: P<0.01.

Supplemental Figure 2. Adjusted effect size (d-Cohen and 95 %CI) and differences in the change in percentage hepatic fat, body mass and composition, cardiometabolic and diabetes risk factors between the control group (children participating in the family based lifestyle and psycho-educational program) and the intensive group (children participating in the multicomponent intervention including lifestyle and psycho-educational program and exercise training). Differences between the control and the intensive intervention groups in terms of the changes in primary and secondary outcomes were examined per protocol by ANCOVA, adjusting for baseline values. *Negative Cohen's d values obtained for the differences in the changes in adiposity and cardiometabolic and diabetes risk between the two groups (in favor of the intensive group) were multiplied by -1 for illustrative purposes. HDL: high density lipoprotein; HOMA: homeostasis model assessment; TG/HDL: triglycerides to high density lipoprotein ratio; LDL: low density lipoprotein; GGT: gamma glutammyl transferase; VO_2 peak: peak oxygen consumption in the treadmill test; 20msrt: performance in the 20 meters shuttle run test.

Supplemental Figure 3. Effect size (d-Cohen and 95 %CI) and differences in the change in percentage hepatic fat, body mass and composition, cardiometabolic and diabetes risk factors between the control group (children participating in the family based lifestyle and psycho-educational program) and the intensive group (children participating in the multicomponent intervention including lifestyle and psycho-educational program and exercise training). Differences between the control and the intensive intervention groups in terms of the changes in primary and secondary outcomes were examined on an intention to treat basis ANOVA (unadjusted P) and ANCOVA adjusting for baseline values (adjusted P). Changes were calculated as post-intervention minus pre-intervention values. Data are means (standard deviation). *Negative Cohen's d values obtained for the differences in the changes in adiposity and cardiometabolic and diabetes risk between the two groups (in favor of the intensive group) were multiplied by -1 for illustrative purposes. HDL: high density lipoprotein; HOMA: homeostasis model assessment; TG/HDL: triglycerides to high density lipoprotein ratio; LDL: low density lipoprotein; GGT: gamma glutamyl transferase; VO2 peak: peak oxygen consumption in the treadmill test; 20msrt: performance in the 20 meters shuttle run test.

Table 1. Characteristics of the children participating in the study.

	Total			Control group (CI)			Intensive group (II)			P
	N	Mean	SD	N	Mean	SD	N	Mean	SD	
Age (years)	116	10.6	1.1	57	10.6	1.1	59	10.5	1.0	0.701
Girls (n, %)	116	62	53.4	57	30	52.6	59	32	54.2	0.862
Non-Spanish origin of the mother (N, %)	116	18	15.5	57	6	10.5	59	12	20.3	0.114
High maternal educational level (N, %)	115	84	73.0	57	46	80.7	58	38	65.5	0.092
Family history of diabetes (N, %)	115	9	7.8	57	7	11.3	58	2	4.0	0.094
Tanner stage (N, %)										
Telarche or gonadarche	108			55			53			0.277
I		38	34.3		20	36.4		22	41.7	
II		30	38.9		20	36.4		12	22.6	
III		19,	15.7		12	21.0		11	20.8	
IV-V		10	11.1		3	5.4		8	15.1	
Pubarche	108			55			53			0.865
I		37	34.3		20, 36.4			17	32.1	
II		42	38.9		22, 40.0			20	37.7	
III		17	15.7		7, 12.7			10	18.9	
IV-V		12	11.1		6, 10.9			6	11.3	
Weight status	116			57			59			0.727
Overweight (N, %)		49	42.3		25	43.9		24	40.7	
Obesity grade I (N, %)		58	50.0		29	50.9		29	49.2	
Obesity grade II (N, %)		6	5.2		2	3.5		4	6.8	
Obesity grade III (N, %)		3	2.6		1	1.8		2	3.4	
High blood pressure (N, %)	116	8	6.9	57	3	5.3	59	5	8.5	0.350
Hypertriglyceridaemia (N, %)	115	9	7.8	57	3	5.3	58	6	10.3	0.490
High TG/HDLc (N, %)	114	37	32.	56	17	30.4	58	20	34.5	0.692
Hepatic steatosis (N, %)	115	41	35.7	57	18	31.6	58	23	39.7	0.239
Insulin resistance (N, %)	114	51	44.7	55	24	42.9	58	27	46.6	0.705

Control group: children participating in the family-based lifestyle and psycho-educational program; Intensive group: children participating in the same program plus the supervised exercise program; TG/HDLc: triglycerides to high density lipoprotein cholesterol ratio.

Table 2. Percentage hepatic fat, body composition, cardiometabolic and diabetes risk factors, and physical fitness before (Pre) and after (Post) participation in the family-based lifestyle and psycho-educational intervention program (control group, CI) or the plus exercise training (intensive group, II) in children with overweight/obesity (*per protocol* analysis).

	Control group (CI)						Intensive group (II)						CI *vs*. II
	Pre			Post		P	Pre			Post		P	P_{Pre}
	N	Mean	SD	Mean	SD		N	Mean	SD	Mean	SD		
Abdominal adipose tissue													
Visceral adipose tissue	50	5.2	2.8	5.2	2.9	0.769	49	5.6	4.5	4.5	3.6	**0.006**	0.836
Visceral adipose tissue L2-L3 (cm^2)													
Fat fraction VAT L2-L3 (%)													
Visceral adipose tissue L3 (cm^2)													
Fat fraction VAT L3 (%)													
Visceral adipose tissue L4-L5 (cm^2)													
Fat fraction VAT L4-L5 (%)													
Subcutaneous adipose tissue													
Subcutaneous adipose tissue L2-L3 (cm^2)													
Fat fraction ASAT L2-L3 (%)													
Subcutaneous adipose tissue L3 (cm^2)													
Fat fraction ASAT L3 (%)													
Subcutaneous adipose tissue L4-L5 (cm^2)													
Fat fraction ASAT L4-L5 (%)													
Subcutaneous adipose tissue L2-L3 (cm^2)													
Fat free abdominal tissue													
Muscular tissue													
Muscular tissue L2-L3 (cm^2)													
Fat fraction AMT L2-L3 (%)													
Muscular tissue L3 (cm^2)													
Fat fraction AMT L3 (%)													**0.010**
Muscular tissue L4-L5 (cm^2)													0.365
Fat fraction AMT L4-L5 (%)													0.284
Psoas (cm^2)													0.229
													0.130

SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure calculated as diastolic pressure + [0.333 × (systolic blood pressure - diastolic pressure)]; HDLc: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; TG/HDLc: triglycerides to high density lipoprotein cholesterol ratio; HOMA: homeostasis model assessment; Gamma-GT: gamma-glutamyl transferase. 20msrt: 20 meters shuttle run test. *Analyzed with log (ln) transformed values, but non-transformed data are shown in table. P indicates statistical differences between Pre and Post values (paired Student t test). P_{pre} indicates statistical differences in baseline values (Student t test). P_{post} indicates statistical differences in Post-intervention values between the control and the intensive intervention groups (Student t test).