Fitness and energy expenditure after strength training in obese prepubertal girls

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ABSTRACT

Purpose: The purpose of this study was to determine the effects of a school-based, low-volume strength training program on energy expenditure, strength, and physical fitness in obese prepubertal girls.

Methods: A longitudinal, 5-month strength training exercise program was undertaken by healthy, obese (>95th percentile weight-for-height, N = 11) girls age 7-10 yr. The following were measured: strength by the one-repetition maximum test; fitness (V·O\textsubscript{2}\text{peak}) by a treadmill exercise test; resting metabolic rate (RMR), 24-h sedentary energy expenditure (SEE), and sleeping metabolic rate (SMR) by room respiration calorimetry; and total energy expenditure (TEE) by the doubly labeled water method. Physical activity energy expenditure (AEE) was calculated as TEE - (RMR + 0.1*TEE) and physical activity level (PAL) as TEE/RMR. An age-matched, nonoverweight control group was measured for V·O\textsubscript{2}\text{peak} and RMR over the same time period.

Results: Strength increased by 19.6 and 20.0% in the upper and lower body (P < 0.01), respectively. V·O\textsubscript{2}\text{peak} (mL·min\textsuperscript{-1}·kg\textsuperscript{-1}) increased in both groups over time (P < 0.05), but not when covaried for fat-free mass (FFM) or weight. After adjusting for FFM or weight, RMR did not change, but SMR and 24-h SEE decreased significantly in the exercise group. There were no changes in nonprotein respiratory quotient or substrate oxidation. No changes in TEE, AEE, and PAL occurred, either unadjusted or adjusted for FFM or weight.

Conclusion: This long-term, school-based, low-volume strength training program favorably increases strength in obese prepubertal girls but does not increase their daily energy expenditure.

The recently released figures from the National Health Examination Survey (18) indicate that overweight prevalence (based on the 85th percentile weight for height) in children and adolescents is 22% in the U.S. The recommendations included focusing on prevention of overweight by increasing physical activity in children and adolescents (18). Early interventions in childhood and adolescence may decrease the risk of the obesity-associated complications apparent in adults.

One potential contributing factor in the development of obesity is reduced energy expenditure (EE). If reduced EE is indeed a factor, increasing EE should protect against the development of obesity. Only a few intervention studies have been conducted in children that have attempted to increase EE using exercise. In one study (1) using doubly labeled water to measure total EE (TEE), obese adolescent boys increased TEE by 12% after an aerobic exercise training program; half of this increase was attributed to the exercise, and the remainder was the result of spontaneous physical activity. In children, no studies have examined the impact of strength training on EE, whereas strength training intervention studies in older men and women have been shown to increase resting metabolic rate (10,15). However, utilizing a 24-h
calorimeter, we reported no changes in 24-h sedentary energy expenditure or sleeping metabolic rate in healthy older women after strength training (15). No studies of which we are aware have specifically examined 24-h EE and TEE and their components, using two state-of-the-art techniques (24-h calorimetry and doubly labeled water) after a strength training exercise intervention in children.

Physical fitness or aerobic capacity, measured by a \( V_{\text{O}_2\text{peak}} \) test, has been shown to be similar between overweight and normal weight children after adjustment for differences in weight or fat-free mass (FFM) (16). Aerobic exercise training studies in children have been shown to increase physical fitness or \( V_{\text{O}_2\text{peak}} \) (6). Whether strength training can alter \( V_{\text{O}_2\text{peak}} \) in children is unclear.

Thus, limited data are available about this type of exercise intervention on these metabolic parameters in prepubertal girls, particularly obese girls. The purpose of this study was to determine the effects of a school-based, low-volume strength training program on strength, physical fitness, and energy expenditure in obese prepubertal girls.

**METHODS**

**Subjects**

Twelve healthy, obese girls were recruited from one elementary school in the Birmingham, Alabama area to participate in the exercise training program. One girl dropped out of the study, so data are presented on 11 children. All girls were in the age range 7-10 yr, Tanner stage I, and were >95th percentile weight-for-height, according to the National Center for Health Statistics (7). Two girls were in Tanner stage II by the end of the study. Tanner stage I is defined as no breast development and no pubic hair, with Tanner stage II defined as breast budding and some pubic hair. Tanner stage was evaluated by a pediatrician. Eleven girls 7-10 yr of age and <95th percentile weight-for-height (mean = 65th percentile) served as controls. Each group included 1 African-American and 10 Caucasian girls. Individuals with cardiovascular disease, anemia, diabetes, significant renal or hepatic disease, hypothyroidism, musculoskeletal problems, those who took medications on a regular basis, or those on special diets were excluded from the study. All participants and their parents provided written informed consent to participate in this study, which was approved by the Institutional Review Board of the University of Alabama at Birmingham (UAB).

**Study Protocol**

Children were admitted to the General Clinical Research Center (GCRC) at the UAB Hospital at 7 a.m., after a 12-h overnight fast. After resting quietly, resting metabolic rate was measured for 30 min on day 1. The children were transported to the Energy Metabolism Laboratory for body composition measures by dual-energy x-ray absorptiometry (DXA) later that day. The children spent 24 h in the GCRC before the 24-h calorimetry measurements and consumed all meals there. The evening before the calorimeter test at approximately 6 p.m., a baseline urine sample was collected, and a doubly labeled water (DLW) dose was given. On day 2, subjects underwent a 24-h calorimeter test. Two urine samples were collected during the morning following dosing (in the calorimeter) for TEE determination. Food was transported to the laboratory from the GCRC. Subjects were fed meals at approximately 8 a.m., 12 p.m., and 5 p.m., with snacks at 10 a.m. and 2 p.m. The children exited the calorimeter the following morning (day 3). Approximately 14 d later, the children returned in the morning for the treadmill test after an overnight fast. The final urine samples for the DLW measurements were then collected (14 d after dosing). After initial metabolic testing, the children underwent three training sessions at school to familiarize themselves with the exercise equipment. After the baseline strength testing, the training group exercised three times each week for 5 months. During the year, the children also participated in their regular physical education classes at school. At the end of the program, the children returned to the GCRC (at least 24 h after the last exercise session) to repeat the above procedures. For the control children, the girls returned for final testing after an overnight fast and completed the RMR test followed by the treadmill test. The children were initially tested in October and November, and posttraining testing was completed in May.

**Body Composition**

Total body composition was assessed by DXA (DPX-L, Lunar Radiation Corp., Madison, WI). For the exercise group, the scan was analyzed using the Adult Software (version 3.62) and for the control group using the Pediatric Software (version 1.5e). The DXA allows for determination of lean tissue mass, fat tissue mass, and bone mineral content. FFM is defined here as the sum of lean tissue mass and bone mineral content. Each subject was asked to lie motionless on a table for approximately 20 min. Data from the DXA scans have previously been reported (17).

\[ V_{\text{O}_2\text{peak}} \]

Fitness capacity was measured by a \( V_{\text{O}_2\text{peak}} \) test. The treadmill protocol involved a constant speed of 2.5 mph at an initial 0% grade for the first 4 min. The average of min 3 and 4 constituted the steady state. The grade was then increased to 10%. Every 2 min thereafter, the grade was increased by 2.5% to a maximum of 22.5%, when speed was increased by 0.6 mph. Exercise measures (\( V_{\text{O}_2} \), ventilation, respiration rate, and heart rate) and respiratory quotient (RQ) were examined during the steady-state period and at peak exercise. \( V_{\text{O}_2\text{peak}} \) was determined by an RQ > 1.0, heart rate > 195 bpm, and volitional fatigue. A Sensormedics 2900 metabolic cart (Yorba Linda, CA) was used to collect the respiratory gases.

**Dietary Analysis**

On the day preceding and during the calorimetry tests, the exercise group was fed a balanced diet. This diet was derived from the American Diabetes Association exchange lists for meal planning, designed to approximate 50% carbohydrate, 30% fat, and 20% protein. The children's energy intake was based on the individual weight (188 kJ·kg\(^{-1}\)·body weight). Intake was adjusted posttraining for the increases in weight over time. The main variables of interest were total caloric intake and percent of calories from carbohydrate, protein, and fat. Food records were analyzed using the Minnesota Database System (Minneapolis, MN) by one individual.

**Energy Expenditure**

**Resting metabolic rate (RMR).** RMR was determined in the morning after a 12-h overnight fast. The subjects reported to the General Clinical Research Center (GCRC) at 7 a.m. After resting quietly for 30 min, RMR was determined using a Deltracrtir system (Sensormedics, Yorba Linda, CA) for 30 min. RMR was calculated using the equation of de Weir (3). With a sample size of 12 subjects per group, we could detect a difference of 502 kJ between each group for the RMR measurements with a power of 0.81.

**Calorimeter measurements of EE and substrate oxidation.** Measurements of 24-h EE and substrate oxidation were taken in a whole room respiration calorimeter. The calorimeter design characteristics and calibration have been previously described in detail (15). Briefly, the room was equipped with a fold-out bed, desk, chair, lamp, refrigerator, toilet, sink, television/VCR, and telephone. Oxygen consumption (\( V_{\text{O}_2} \)) and carbon dioxide production (\( V_{\text{CO}_2} \)) were continuously measured by the
magnetopneumatic differential $O_2$ analyzer (Magnos 4G) and the NDIR industrial photometer differential CO$_2$ analyzer (Uras 3G, both Hartmann & Braun, Frankfurt, Germany). The calorimeter was calibrated before each subject entered the chamber. Based on duplicate studies in 19 children, the average coefficient of variation for 24-h EE was 5.6% and for 24-h RQ was 2.0% (R. Figueroa-Colon, unpublished observations).

The subjects were transported from the GCRC to the Energy Metabolism Laboratory in the Nutrition Sciences Building at 7 a.m. The subjects entered the calorimeter at 8 a.m. During the stay in the calorimeter, each subject was not allowed to exercise, although freedom of movement was permitted at all times during the day. The subject was awakened the following morning (6:30 a.m.). The subject exited the room after 23 h in the chamber, allowing time for calibration before the next subject entered. Sleeping metabolic rate (SMR) and 24-h sedentary energy expenditure (SEE) were calculated by the de Weir equation (3). All measures of EE were extrapolated over 24 h and expressed as kJ·d$^{-1}$. For the purposes of this study, SMR was determined by averaging EE for the time when the child went to sleep until she was awakened. Protein oxidation was determined from 24-h urinary urea nitrogen excretion. Carbohydrate and fat oxidations were calculated from the 24-h nonprotein respiratory quotient (nPRQ) and expressed as percentages of nonprotein EE or NPEE (4). For the post-training assessments, the children entered the calorimeter at least 48 h after the last exercise session.

**Free-living energy expenditure.** DLW was used to measure TEE over a 14-d free-living period in the exercise group. A baseline urine sample was collected in the evening at the GCRC before the 24-h calorimetry and was followed by oral administration of doubly labeled water at approximate doses of 0.15 g of H$_2^{18}$O and 0.12 g of H$_2$O per kg of body mass. A total of four timed urine samples were collected postdose. Two were collected in the morning following dosing (in the calorimeter), with the overnight period assuring isotopic equilibration. Two urine samples were collected in the morning 14 d later. This protocol minimizes error owing to diurnal variation in isotope turnover (13) while reducing the effects of analytical error. Samples were analyzed in triplicate for H$_2^{18}$O and H$_2$O using a Fisons-VG Optima isotope ratio mass spectrometer (VG Ltd., Manchester, England). The average standard deviation for triplicate analysis in a study using the sample preparation procedures was approximately 4 dels/mille for deuterium and 0.2 dels/mille for $^{18}$O at the Energy Metabolism Research Unit in Birmingham.

Turnover rates and zero-time dilution spaces of H$_2^{18}$O and H$_2$O were calculated from the slope and intercept of the regression line between the natural logarithm of isotopic enrichment in urine and time after dosing, as previously described (5). The dilution space ratios were 1.0466 ± 0.007 and 1.0654 ± 0.013 in the obese girls before and after training, respectively. These dilution space ratios were significantly different from each other. The mean of each was used for all calculations. Several other laboratories report dilution space ratios above 1.045 as previously reviewed by Speakman et al. (14). CO$_2$ production rate was calculated according to the equation R2 of Speakman et al. (14), which is a modification of equation A6 of Schoeller et al. (12), based on post hoc evaluation of the group mean deuterium: oxygen-18 dilution space ratio. The equation that was used to perform the DLW calculations (Speakman R2) takes into account potential group differences in the dilution space ratio and has been shown to improve the accuracy and precision of the DLW technique. CO$_2$ production rates were converted to EE using the de Weir equation (3) using the food quotient of the diet based on the child's diet composition. Accurate measurement of the food quotient is not critical for interpretation of DLW data, as TEE estimates will be in error by only 1% for 0.01 unit error in food quotient. Total EE was calculated with the de Weir equation (3). Assuming that $^{18}$O was because of the thermic effect of food, physical activity EE was calculated as TEE - (RMR + 0.1*TEE). Physical activity level (PAL) was calculated as TEE/RMR.

**Strength Assessment**

Before the strength tests, the exercise group was allowed to become familiar with the equipment and exercise techniques. This included instruction on each of the machines followed by watching each child complete the exercise. Strength was assessed only on the bench press and leg press by the one-repetition maximum (1-RM) test, defined as the maximum amount of weight that could be lifted successfully one time. Starting with a weight used in the preliminary sessions, the subjects attempted lifts with gradually increased weights (-10% at first, decreasing to 5% and 2.5% as difficulty became evident). Successful attempts were made with a 90-s rest period between attempts until failure occurred. Test-retest reliability in our laboratory of 1-RM testing and isometric testing varies from 0.95 to 0.99 depending on the type of 1-RM test (-5% variation in all tests). Approximately three to five trials were needed to reach the 1-RM both before and after training. Isometric strength of the knee extensors was also tested at a knee position of 110° flexion while each subject was seated. Movement of the hips was prevented by strapping the subject to the chair at the thighs and torso. Force was measured using a Universal Shear Beam Load Cell (model LCC 500, Omega, Stamford, CT). A Digital Transducer (Omega, Stamford, CT) gave instantaneous force feedback to the subjects. Two maximal isometric contractions were recorded after four practice trials. The average of the values was used for statistical analyses.

**Strength Training**

Strength training took place in 20-min sessions, three times each wk for 5 months at the elementary school. Each session included a whole-body strength training program, with the subjects completing six upper-body exercises and one lower-body exercise using a Paramount circuit training apparatus (Paramount Fitness Equipment Corp., Los Angeles, CA). The order of exercises was leg press, bench press, military press, bicep curl, latissimus pull down, tricep extension, and sit-ups. All subjects completed two sets of 12 repetitions for the upper-body exercises and two sets of 15 repetitions for the leg press. Each exercise took approximately 45 s to complete with a 45-s rest period between exercises. Subjects started the training program at 50% of 1-RM for the bench press and leg press with the other four exercises at the lowest weight on the machine. After three sessions, the weight was gradually increased in the smallest increments until the subjects could complete in strict form two sets of 12 or 15 repetitions for a given exercise. To maintain the appropriate intensity (70% of 1-RM) for the prescribed repetitions, adjustments in weights were made approximately every 2 wk initially and then as necessary to continue to promote increases in strength. Attendance was taken at each exercise session (total number of sessions = 61) to monitor compliance with the program. There were breaks in the training program during the winter holidays and 1 wk during the spring. Subjects were monitored by an exercise physiologist and at least one graduate student.

**Statistical Analysis**

All variables were compared from baseline values to final values using paired t-test. For those variables where appropriate, FFM or weight was used as a covariate (ANCOVA procedure) to normalize the data. Repeated measures ANOVA was used for variables that were assessed in both the exercise and control groups. All data were analyzed by SAS for Windows (Cary, NC) with significance set at $P < 0.05$.

**RESULTS**

**Subject Characteristics**

Eleven children in the exercise group completed the posttraining testing for strength and $V_O^2$peak, and 10 completed the 24-h calorimetry and TEE studies (Table 1). Compliance with the training in the exercise group was 83%, although compliance during the last month was 70% because they took school state achievement tests.
Eleven children in the control group returned for body composition, RMR, and fitness assessments.

### Table 1. Subject characteristics in prepubertal girls.

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<th>Exercise Group</th>
<th>Control Group</th>
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<td>Baseline</td>
<td>Final</td>
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| **Age (yr)**          | 8.7 ± 0.7 | 9.2 ± 0.8
|                      | (N = 11) |       |          |       |
| **Height (cm)**       | 136.0 ± 6.5| 138.3 ± 6.7 | 130.3 ± 5.4| 134.3 ± 5.5 |
|                      |          |          |          |       |
| **Weight (kg)**       | 46.6 ± 9.4| 50.6 ± 10.3 | 29.1 ± 2.8 | 32.0 ± 3.0
|                      |          |          |          |       |
| **Percent fat (%)**   | 38.9 ± 6.6 | 39.2 ± 5.9 | 28.1 ± 4.8 | 28.7 ± 4.8 |
|                      |          |          |          |       |
| **FFM (kg)**          | 27.1 ± 3.2 | 29.2 ± 3.6 | 20.2 ± 2.1 | 22.1 ± 2.2 |

Values are expressed as mean ± SD.

*Significantly different from baseline value (P < 0.001).

# Significantly different from baseline value (P < 0.05).

*Significantly different between groups (P < 0.05).

aFFM, fat-free mass (the sum of lean mass and bone mineral content).

There were no significant interactions for age, weight, height, fat mass or FFM between groups (Table 1). Age and weight (P < 0.001) and height (P < 0.05) significantly increased over the 5-month period in both groups. The DXA scan revealed significant increases in FFM over time in both groups (P < 0.001). There were significant differences between the two groups for weight, height, % body fat, and FFM (P < 0.05).

### Strength

There was a 19.6% increase in 1-RM bench press, and a 20.0% increase in 1-RM leg press (P < 0.01) in the exercise group (Fig. 1). Isometric strength of the knee extensors increased by 35.2% (P < 0.001).

### VO\textsubscript{2peak}

There were no significant changes over time or between groups for steady-state ventilation, respiration rate, or VO\textsubscript{2} (Table 2). However, steady-state HR decreased significantly in each group (P < 0.05). Steady-state RQ significantly decreased in the control group but increased slightly (nonsignificant) in the exercise group over time. The ratio of steady-state VO\textsubscript{2} to VO\textsubscript{2peak} significantly decreased from before to after training in the exercise group (P < 0.05). There were no significant changes for peak heart rate, ventilation, respiration rate, or RQ between groups or over time. VO\textsubscript{2peak} (mL·min\textsuperscript{-1}) significantly increased over time in both the exercise and control groups (P < 0.05) but not when using ANCOVA with FFM or weight as covariates. There were no changes in time to exhaustion on the treadmill.
Energy Intake in the Calorimeter

There were no significant differences in the percent of calories from protein (13 ± 3 vs 12 ± 2%), carbohydrate (63 ± 4 vs 63 ± 3%), or fat (26 ± 3 vs 27 ± 2%) before versus after training in these children, respectively, for the day in the calorimeter. Thus, the food quotients of the diets were identical (0.92 ± 0.01). Energy intake for the calorimeter before training (8966 ± 2180 kJ·d⁻¹) was significantly less than after training (10,088 ± 2059 kJ·d⁻¹) (**P < 0.01, Table 3**). The children increased their weight over the 5 months, and the calorimeter energy intake requirement was based on body weight. Therefore, the posttraining intake was calculated with this increased weight and was consequently higher. Although the composition of the diet was held constant from before to after training as mentioned above, the increased intake was significantly higher for protein and carbohydrate (expressed in grams) after training. The difference in fat intake in grams from before to after training approached significance (**P = 0.07**).

Energy expenditure and substrate oxidation in obese, prepubertal girls at baseline before training and final values after training. (Table 3)

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<th>Baseline</th>
<th>Final</th>
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| 24-h respiration calorimetry | 24-h sedentary EE in the calorimeter significantly decreased (**P < 0.05**, Table 3). These differences remained significant after adjusting for FFM (9163 to 7489 kJ) or weight (8908 to 7745 kJ). The npRQ did not change from before to after training, and consequently neither did carbohydrate or fat oxidation expressed as a percentage of nonprotein EE (Table 3).

### Discussion

Owing to the increasing prevalence of overweight in children and adolescents (18), increased physical activity has been recommended. Intervention studies...
designed to increase EE in obese prepubertal children have been few in number. Therefore, we designed a strength training intervention study in a school-based setting to measure these parameters in obese children. This study indicates that a school-based, low-volume strength training program in obese prepubertal girls can increase strength but does not significantly improve energy expenditure. Over time, the increases in fitness and RMR are the result of changes in body composition or growth, as evident from the data from each group. It should be noted that the purpose of our study was to determine for practical purposes whether training conducted in a school and of relatively low volume would promote positive changes in energy metabolism; this is not to say that a higher volume, more intense strength training program might not alter energy expenditure.

As illustrated in Fig. 1, obese girls gain strength consequent to this type of training. Strength gains in this study were on the order of 20% for the 1-RM bench press and leg press, with a 35% increase in the isometric strength test. Other studies have reported significant increases in strength of similar magnitude in normal weight, prepubertal boys and girls after isotonic strength training (2,9,11). Our study has one advantage of utilizing the 1-RM technique to assess strength changes. Kraemer et al. (8) pointed out that in children, muscle strength is rarely evaluated using the classic 1-RM testing methods on the equipment used in the training. Our training program began at 50% of 1-RM and progressed to 70% of 1-RM, which is within the guidelines recommended (8). The effectiveness of strength training in children seems to be dependent on the intensity of the loading and to maintain these strength gains; more than one high-intensity strength training session per week is required (8). Proposed mechanisms for the improvement in strength include improved motor skill coordination, increased motor unit activation, and other undetermined neurological adaptations (11).

Submaximal exercise measures of heart rate, ventilation, respiratory rate, and V̇O₂ were not affected by the exercise training. In the control group of prepubertal girls, there were also no changes in the submaximal measures. Over time, cardiorespiratory fitness (V̇O₂peak), expressed in mL·min⁻¹, increased in both groups. No other variables measured at peak exercise changed. The V̇O₂peak increases over time were 14.4% in the exercise group and 17.4% in the control group. However, when covaried for FFM or weight, there were no significant differences in V̇O₂peak from before to after training or from initial to final values. Both groups reached peak capacity during the test; as indicated by the peak RQ and heart rate. Improvements in V̇O₂ 2peak (19.4% expressed in L·min⁻¹, 13.8% expressed in mL·kg⁻¹·min⁻¹) have been observed previously in prepubertal boys after 14 wk of resistance training (19).

There was an increase in RMR over time in both the exercise and control groups. When the data were adjusted for the increases in weight and FFM that occurred, no significant effects were observed. Therefore, the increase seems to be the result of the changes in FFM and weight, which occur with advancing age and growth.

Sleeping metabolic rate also did not change in the training group but decreased when covaried for FFM or weight. The decreases in 24-h sedentary EE and sleeping metabolic rate are difficult to explain. The protocols were identical in terms of time spent sleeping, eating, etc. from before to after the training. Because the children increased their body weight and FFM, one would expect a slight increase in the unadjusted 24-h sedentary EE. For the calorimeter studies, it seems likely that the children were less enthusiastic about remaining 24 h in a calorimeter, and boredom occurred, contributing to greater television watching and less movement in the calorimeter. Unfortunately, we did not have motion sensors in the calorimeter to detect whether the children were more sedentary. However, our observation of the children would suggest that this occurred. As for the free-living measurements, the TEE also decreased, but not significantly. These results are also surprising because anecdotal evidence from the families indicated that the children were more active. When TEE was adjusted for FFM or weight, it also did not significantly change, but the adjusted values were higher after training. This might indicate that the children were indeed more active. In agreement, although AEE and PAL remained unchanged, the adjusted AEE also tended to increase after training. No studies of which we are aware have measured energy expenditure after a strength training program in children, so these findings are noteworthy.

It should be noted that the postraining measurements for TEE were taken while the children were still enrolled in the exercise program, as we felt it was necessary to continue the training for the DLW measures. However, this is unlikely to have altered the values considerably because the children were only able to attend four sessions during the TEE measurement period. This might account for some of the increase in TEE when the data were covaried for FFM and weight. We also did not restrict the children from participating in other physical activities during the school year, and several of the girls were involved in outside activity programs at the start of the study and not the end of this study. This may have influenced our findings. Compliance in our study was good; however, we did have reduced compliance the last month of the training program owing to the children taking school achievement test at the start of the school year and of relatively low volume would promote positive changes in energy metabolism; this is not to say that a higher volume, more intense strength training program might not alter energy expenditure. In conclusion, obese prepubertal girls demonstrate gains in strength but do not significantly increase their energy expenditure after undergoing a long-term, school-based, low volume strength training program. Future research should focus on interventions combining aerobic and strength training exercises to increase both energy expenditure and strength, and consequently improve overall metabolism in obese children.

REFERENCES


Key Words: CALORIMETRY; METABOLISM; DOUBLY LABELED WATER