Reduction in Butyrylcholinesterase Activity and Cardiovascular Risk Factors in Obese Adolescents after 12-Weeks of High-Intensity Interval Training

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¹Department of Physical Education, Federal University of Paraná, Curitiba, Paraná, Brazil, ²Department of Physical Education, University Paranaense, Francisco Beltrão, Paraná, Brazil, ³Polymorphism and Linkage Laboratory, Department of Genetics, Federal University of Paraná, Curitiba, Paraná, Brazil, ⁴Department of Physical Education, State University of Maringa, Maringá, Paraná, Brazil

ABSTRACT

Pizzi J, Furtado-Alle L, Schiavoni D, Lopes WA, Silva LR, Bono GF, Souza RLR, Leite N. Reduction in Butyrylcholinesterase Activity and Cardiovascular Risk Factors in Obese Adolescents after 12-Weeks of High-Intensity Interval Training. JEPonline 2017;20(3):110-121. The purpose of this study was to evaluate the effect of 12 wks of high-intensity interval training (HIIT) on butyrylcholinesterase (BChE) and cardiovascular risk factors in obese adolescents. The subjects were 54 obese adolescents, divided into two groups: the high-intensity interval training group (HIITG, n = 20) and the control group (CG, n = 34). The HIIT resulted in a significant decrease in BMI z-score, WC, TC, LDL-C, and BChE activity (P<0.05). The CG showed a significant increase in BChE (P = 0.05) with no change in BMI, BMI z-score, CC, TC, LDL-C, HDL-C, and TG. The decrease in BChE activity with HIIT training was accompanied by the decrease in biochemical markers, thus indicating that the BChE activity can be used as a secondary marker for cardiovascular risk factors that are associated with child and adolescent obesity.

Key Words: Exercise, Metabolism, Obesity, Youth
INTRODUCTION

The rise in global obesity rates challenges healthcare professionals to revisit the treatments used to decrease excess weight in all age groups (23). The main concern is not obesity itself, but associated cardiovascular risk factors and its negative psychosocial impacts in children, adolescents, and adults (42). One of the treatments for overweight, consists in concomitant nutritional education and physical activity (31), which may include different modalities and exercise intensities (37).

Lipid disorders are related to poor diet (34) and lack of physical activity (16) as well as genetic factors (33). The enzyme butyrylcholinesterase (BChE) has been linked to lipid metabolism and some risk factors for cardiovascular disease such as obesity (13, 19) and hypertension (5). The activity of BChE is higher in obese than in eutrophic (6, 19), probably due to the role of BChE on the hydrolysis of choline esters, which are products of the free fatty acids metabolism and liver lipogenesis (13).

The regular practice of aerobic exercise decreases BChE activity in obese adolescents, thus bringing it closer to the values observed in non-obese adolescents. This suggests that aerobic exercise can lead to physiological regulation of BChE activity in plasma that is correlated to positive changes in serum lipid concentrations (3). However, other types of exercises have not been assessed for BChE activity.

The high-intensity interval training (HIIT) has been used in healthy young people to promote the control of body weight, improvement in maximal oxygen uptake (30), insulin sensitivity (4), and a more effective and time-efficient intervention for improving blood pressure and aerobic capacity levels in obese youth in comparison to other types of exercise (20). Regarding the lipid profile, some studies have indicated that HIIT decreases total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and triglycerides (TG) in children and adolescents (36), while having no effect on others subjects (2). This finding is confusing and needs clarification (26).

Considering that HIIT can be a therapeutic alternative for patients with obesity and risk factors for cardiovascular disease, the purpose of this study was to evaluate changes in BChE activity and cardiovascular risk factors after 12 wks of HIIT in obese adolescents.

METHODS

Subjects
The sample consisted of 54 obese children and adolescents from Southern Brazil (Francisco Beltrão/Paraná) who were placed into either the high intensity interval training group (HIITG, n = 20) or the control group (CG, n = 34) for a period of 12 wks. The inclusion criteria were: (a) declared to be in good health (were not using drugs or under treatment for any disease); (b) between 10 and 15 yrs of age; (3) regularly enrolled in school; (4) diagnose as being obese; and (5) Informed Consent form was signed by parents or guardians. The exclusion criteria were: (a) the presence of musculoskeletal problems or physical disability that does not allow participation in physical activities; (2) did not participate in the second physical evaluation of the study; and (3) attended 80% of training sessions. Before being included in the study, all subjects underwent a medical assessment with detailed history and physical
examination. Those responsible for the subjects were informed and signed a consent term that was previously approved by the Ethics Committee.

**Procedures**

Standing height was measured to the nearest 0.1 cm using a portable stadiometer coupled to a digital scale with resolution of 100 g, which was used to measure body weight. Body mass index (BMI) and BMI z-score were calculated as growth curves using the Anthro Plus program (43). An anthropometric tape of inextensible metal with a resolution of 0.1 cm was used for the measurement of waist circumference (WC) (27). The pubertal development stages were determined by a physician who used the self-assessment of pubic hairiness (P1-P5) (32) to identify the maturational stage of the subjects. Blood samples were collected in the morning after 12 hrs of fasting for analysis of TC, high density lipoprotein cholesterol (HDL-C), and TG. The LDL-C in mg·dL⁻¹ was estimated using the Friedewald formula (18).

BChE Activity Assay was performed according to Boberg et al. (6). Its principle is the hydrolysis of propionyl thiocholine by butyrylcholinesterase, producing propionic acid and thiocholine, which reacts with DTNB (5,5'-bisditio-2-nitrobenzoic acid) to yield 5-thio-2-nitrobenzoate yellow. The BChE activity was measured with a spectrophotometer (423 nm) in both groups at the start of the program and at the end of the 12 wks of the program of physical activities.

The exercise program was conducted independently of school curricular activities of the subjects. Each session consisted of warm-up exercises, running/walking on a sports court at different intensities totaling 45 min with cooling. The exercises were performed in the afternoon 3 times·wk⁻¹ every other day for 12 wks. The interval training protocol consisted of two high-intensity series repeated for 30 sec with the subjects in the HIITG running as fast as they could run interspersed with a recovery period of an active 60 sec walk with 4 min of rest between series. The progression of the training was carried out by adding extra time to run/walk and by decreasing the active recovery from 60 sec to 45 sec and then to 30 sec as the weeks passed (Table 1).

**Table 1. Training Program.**

<table>
<thead>
<tr>
<th>Week</th>
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<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4-5</td>
<td>6-9</td>
</tr>
<tr>
<td>Series</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>2x</td>
<td>2x</td>
<td>2x</td>
<td>2x</td>
</tr>
<tr>
<td></td>
<td>4x30sec/60sec</td>
<td>5x30sec/60sec</td>
<td>6x30sec/60sec</td>
<td>7x30sec/60sec</td>
<td>8x30sec/45sec</td>
</tr>
<tr>
<td>Intensity</td>
<td>100%/50%</td>
<td>100%/50%</td>
<td>100%/50%</td>
<td>100%/50%</td>
<td>100%/50%</td>
</tr>
<tr>
<td>Rest</td>
<td>4 min</td>
<td>4 min</td>
<td>4 min</td>
<td>4 min</td>
<td>4 min</td>
</tr>
</tbody>
</table>

The interval training protocol consisted of two series of high-intensity repeated for 30 sec at 100% speed peak effort (associated maximum heart rate after the progressive cardiac maximum test) interspersed by a period of active recovery 60 sec at 50% of peak velocity with a 4-min rest between series.
Statistical Analyses

Data were analyzed using the Shapiro-Wilk normality test and non-parametric tests for comparisons of the independent (Mann-Whitney) and dependent (Wilcoxon Signed Ranks) variables. The effect size was evaluated as suggested by Cohen (15): 0.20 as small, 0.50 as medium, and 0.80 as major effect. In the present study, values lower than 0.20 were considered as probably trivial, between 0.20 and 0.39 as benefit possible, between 0.40 and 0.79 as benefit, and greater than 0.80 as benefit likely. Chi-square test ($\chi^2$) or Fisher's exact test were used to check for differences in the proportions of subjects with a lipid profile classified as normal or altered in HIITG and CG. Multiple regression analysis was performed to evaluate the independent effect of variables on BChE activity. The level of significance was set at P<0.05.

RESULTS

The general characteristics of the HIITG and CG at baseline are presented in Table 2. There were no dropouts during the 12 wks of the HIIT intervention. All subjects met at least 90% of the training sessions. Although during the initial phase of the study 5 obese adolescents in the HIITG (25%) reported joint discomfort in the lower limbs (knee and ankle), adherence to the HIIT intervention was 100%.

Table 2. Baseline Values of Anthropometric and Biochemical Variables in CG and HIITG. (Mean ± SD; *P<0.05).

<table>
<thead>
<tr>
<th>Variables</th>
<th>CG</th>
<th>HIITG</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>14.29 ± 1.84</td>
<td>12.18 ± 1.55</td>
<td>0.037*</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.63 ± 0.10</td>
<td>1.56 ± 0.12</td>
<td>0.111</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>79.35 ± 15.84</td>
<td>72.28 ± 21.84</td>
<td>0.342</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>29.39 ± 4.20</td>
<td>28.73 ± 4.44</td>
<td>0.051</td>
</tr>
<tr>
<td>BMI (score-z)</td>
<td>2.39 ± 0.79</td>
<td>2.66 ± 0.59</td>
<td>0.837</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>94.70 ± 11.15</td>
<td>96.05 ± 13.18</td>
<td>0.058</td>
</tr>
<tr>
<td>TC (mg·dL⁻¹)</td>
<td>157.71 ± 33.23</td>
<td>177.40 ± 36.25</td>
<td>0.667</td>
</tr>
<tr>
<td>HDL-C (mg·dL⁻¹)</td>
<td>54.19 ± 11.44</td>
<td>52.60 ± 9.88</td>
<td>0.001*</td>
</tr>
<tr>
<td>LDL-C (mg·dL⁻¹)</td>
<td>83.15 ± 21.80</td>
<td>108.24 ± 27.42</td>
<td>0.299</td>
</tr>
<tr>
<td>TG (mg·dL⁻¹)</td>
<td>100.72 ± 74.27</td>
<td>82.75 ± 35.29</td>
<td>0.004*</td>
</tr>
<tr>
<td>BChE (kU·L⁻¹)</td>
<td>6.250 ± 2.084</td>
<td>8.351 ± 2.470</td>
<td>0.037*</td>
</tr>
</tbody>
</table>
The proportion of pubescents and post-pubescents between boys and girls was similar ($\chi^2 = 2.385, P = 0.122$). The chronological age was lower in the HIITG compared to the CG ($P = 0.0000$). In the initial phase, the HIITG had a lower mean height ($P = 0.037$) and higher means of plasma concentrations of LDL-C ($P = 0.001$) and plasma BChE activity ($P = 0.004$) compared to the CG. The proportion of subjects with above than adequate values were similar in the HIITG and the CG for CT ($\chi^2 = 2.29; P = 0.13$), HDL ($\chi^2 = 0.01; P = 0.905$) and TG ($\chi^2 = 1.53; P = 0.216$). A higher proportion of subjects with increased LDL-C was found in the HIITG compared to the CG (Fisher's Exact Test, $P = 0.046$) (Table 2). After 12 wks, the HIITG reduced the BMI z-score, WC, TC, HDL-C, LDL-C and the activity of BChE, but TG did not change. The CG showed increased activity of BChE ($P = 0.005$) and did not change the BMI, BMI z-score, WC, TC, LDL-C, HDL-C, and TG. In the HIITG and CG, the proportions of individuals with high levels of TC ($\chi^2 = 0.08; P = 0.779$), HDL-C ($\chi^2 = 0.01; P = 0.932$), TG ($\chi^2 = 0.26; P = 0.609$), and LDL-C (Fisher's exact test, $P = 0.474$) were similar.

Both groups showed an increase in height and a decrease in body weight after 12 wks. Considering that height is subjected to natural changes of growth, the effect size was not calculated. The response of body weight ($d = 0.14$) was unclear after 12 wks of training. The trend of changes in the variables LDL-C ($d = 0.62$), TC ($d = 0.56$), BMI z-score ($d = 0.59$), WC ($d = 0.48$), and BChE ($d = 0.41$) as a result of training was possibly beneficial for subjects. There were trivial differences in BMI ($d = 0.36$) and TG ($d = 0.32$) (Table 3).

Table 3. Anthropometric and Biochemical Variables in the Initial Stage and After 12 Weeks in the CG and HIITG. (Mean ± SD; *P<0.05; 'Cohen’d)

<table>
<thead>
<tr>
<th>Variables</th>
<th>CG (n = 34)</th>
<th>HIITG (n = 20)</th>
<th>Inference Practice¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.63 ± 0.10</td>
<td>1.65 ± 0.10</td>
<td>0.000* Benefit</td>
</tr>
<tr>
<td><strong>Body Mass (kg)</strong></td>
<td>79.35 ± 15.84</td>
<td>80.31 ± 16.17</td>
<td>0.021* Benefit</td>
</tr>
<tr>
<td><strong>BMI (kg·m⁻²)</strong></td>
<td>29.39 ± 4.20</td>
<td>29.60 ± 4.31</td>
<td>0.166 Benefit Possible</td>
</tr>
<tr>
<td><strong>BMI (score-z)</strong></td>
<td>2.39 ± 0.79</td>
<td>2.34 ± 0.83</td>
<td>0.147 Benefit Possible</td>
</tr>
<tr>
<td><strong>WC (cm)</strong></td>
<td>94.70 ± 11.15</td>
<td>95.62 ± 11.81</td>
<td>0.122 Probably trivial</td>
</tr>
<tr>
<td><strong>TC (mg·dL⁻¹)</strong></td>
<td>157.71 ± 33.23</td>
<td>153.39 ± 33.17</td>
<td>0.305 Probably trivial</td>
</tr>
<tr>
<td><strong>HDL (mg·dL⁻¹)</strong></td>
<td>54.19 ± 11.44</td>
<td>53.23 ± 16.92</td>
<td>0.467 Probably trivial</td>
</tr>
<tr>
<td><strong>LDL-C (mg·dL⁻¹)</strong></td>
<td>83.15 ± 21.80</td>
<td>79.18 ± 31.35</td>
<td>0.0412 Benefit</td>
</tr>
<tr>
<td><strong>TG (mg·dL⁻¹)</strong></td>
<td>100.72 ± 74.27</td>
<td>99.18 ± 48.98</td>
<td>0.900 Probably trivial</td>
</tr>
<tr>
<td><strong>BChE(kU/l)</strong></td>
<td>6.250 ± 2.084</td>
<td>6.697 ± 1.784</td>
<td>0.005* Benefit possible</td>
</tr>
</tbody>
</table>
After 12 wks, the CG showed higher frequency of subjects who increased WC ($Z = -2.390; P = 0.017$), BMI z-score ($Z = -3.194; P = 0.001$), BChE ($Z = -1.979; P = 0.048$), LDL-C ($Z = -3.529; P = 0.000$), and TC ($Z = -3.019; P = 0.003$) compared to the HIITG. The HIITG showed higher frequency of subjects with reduced BChE ($Z = -2.795; P = 0.005$) compared to the CG.

According to the results of the regression models, the TC explained 21% of the variations in the BChE ($\beta = 0.464, P<0.001$). Correcting for age and sex, it was the factor that most influenced the BChE activity. It is noteworthy that the concentration of TC reduced after 12 wks of intervention in the HIITG, and this variation was considered beneficial when calculating the effect size.

**DISCUSSION**

**Anthropometric and Body Composition Measures**

BChE activity is related to the metabolism of lipids (6,35). The increase in BChE is associated with excess weight as well as the accumulation of visceral and endothelial fat (25). The increasing prevalence of overweight and its complications, both in childhood and in adult life, lead to the loss of healthy years of life that is linked to an increase in health care costs (38). Therefore, obesity therapy is an important challenge in health care, particularly since the adherence to treatment and weight loss is often a difficult goal to reach (10).

Aerobic exercise training is generally prescribed as therapy for the obese population (28). It is also prescribed as a means to reducing the associated comorbidities such as hypertension and type 2 diabetes mellitus. In addition to use of aerobic training as exercise medicine, concurrent training (12) and resistance training (1) have also been prescribed in the treatment of obesity. More recently, HIIT has been proposed as a therapeutic strategy to decreasing cardiovascular disease in obese children and adolescents (14,26).

In the present study, 12 wks of HIIT resulted in a decrease in BChE activity, BMI z-score, WC, TC, LDL-C, and an increase in HDL-C while the CG showed an increase in BChE. In agreement, Milano et al. (35) also reported a decrease in BChE activity along with a decrease in TG, LDL-C, BMI, and WC in obese adolescents after 12 wks of aerobic exercise of moderate intensity. Aerobic exercise appears to be an excellent means to decreasing body fat (41), which probably explains the reduction in BChE activity seen in the present study (given that it is associated with excess adipose tissue) (6). In view of this, a likely mechanism for the reduction of BChE with aerobic exercise is the total energy expenditure per session that reaches the threshold for change in fat and lipid metabolism. Also, it is likely that the weekly frequency is more important than just the volume or intensity of training in isolation.

It is important to point out that the present study evaluated adherence in the obese population because HIIT was initially directed to just athletes and healthy adults (21). Yet, the obese children and adolescent subjects' practice of HIIT for 12 wks without dietary intervention showed 100% participation.

One of the features of HIIT is to introduce lower intervention volume, making it effective strategy for accumulating the necessary physiological adjustments and the related health benefits compared to the traditional aerobic exercise programs (7). Time spent in vigorous physical exercise was associated with several positive health outcomes, suggesting that high
intensity activities can help young people reduce the risk of several chronic diseases (11). On the other hand, more research is needed to assess the side effects of intense activity in obese individuals, such as those related to the musculoskeletal system (3). In the present study, 25% of the obese adolescents were found to experience joint discomfort in the lower limbs (knee and ankle), early in the HIIT without reductions in growth in stature as in other studies conducted in adolescents (9).

Regarding the changes in anthropometry after 12 wks of HIIT, there were no significant decreases in body weight, BMI z-score, and WC. While the CG showed increased BMI and WC. Similar results were found in the anthropometric variables in Araújo et al. (2) with obese children when compared to resistance training with HIIT. However, in the study of Buchan et al. (8) in 7 wks of HIIT compared to the CG, there were no changes in the anthropometric variables in the HIITG. Instead, there were only significant increases in WC the CG. Perhaps, this explains the major changes in the biochemical and anthropometric parameters in the present 12-wk study. Also, this point suggests that further investigation in relation to the intervention time is required.

Tjønna et al. (40) reported significant changes in body mass and BMI z-score in both HIITG and interval training of moderate intensity decrease (MIIT) were found in a study with obese female adolescents, although the subjects' WC was significantly decreased only in the HIITG. Also, Lau et al. (24) used HIIT an effective and time-efficient intervention for improving body composition, functional walking, and aerobic endurance in overweight children after 6 wks of training.

**Metabolic Parameters**

After 12 wks of HIIT there were significant decreases in total cholesterol and LDL-C in the HIIT group (with effect size considered as possibly beneficial). Perhaps, the highest average values and changed proportions of LDL-C in the early HIITG may have contributed to the best answer regarding exercise. But, this result should be analyzed with caution because it is a variable that is calculated from the concentrations of other lipid profile components. Although it is noteworthy that the average values of HDL-C decreased in the subjects of the present study, the values were nonetheless normal according to the Guidelines (22). The CG showed no changes in TC, LDL-C, HDL-C, and TG.

Similarly, Lau and colleagues' (24) research on obese adolescents, who examined the effects of HIIT versus MIIT also found significant decreases in TC and LDL-C in response to HIIT. In intra-group comparison, TC, TG, HDL-C, and LDL-C showed significant changes in the HIITG while the MIIT group showed significant changes only in HDL-C and LDL-C. The control group showed no changes (24). However, research that compared aerobic endurance training with HIIT found no differences in the subjects' lipid profile (TC, HDL-C, LDL-C, and TG) after 12 wks, indicating similar results in both types of exercise in obese children (2).

For other types of exercise such as a combination of aerobic exercise and resistance training, the lipid profile did not change after 12 wks (29). Another study using 20 wks of concurrent training showed significant increases in TC and TG, but no changes in HDL-C (17). Similar results for TC and LDL-C were found in the present study, even with an intervention of a shorter duration.
Studies in adults have found positive correlations between the activity of BChE and type 2 diabetes mellitus and TC, TG, and BMI. BChE activity in obese adults is higher than in non-obese (6), which may be related to the greater availability of free fatty acid, which is characteristic of obesity.

With respect to the multiple linear regression in the present study, TC was the primary contributor to the activity level of BChE, which was significantly decreased only in the HIITG. This finding suggests regulation of the physiological activity of BChE in the plasma; whereas, early on individuals in the intervention group had higher values of BChE activity than the CG. Thus, increased activity of BChE in obese individuals as reported in several studies may be a result of metabolic imbalance caused by obesity of which its activity can be reduced by improving the metabolism and physiological conditions via regular exercise. The impact of increased BChE together with increased body weight and obesity indicators suggest that BChE may be more sensitive to changes in body composition, and may even precede changes in the lipid profile. Therefore, it can be considered as an obesity marker in adolescents that seems to respond to regular practice of HIIT.

Limitations of this Study

One limitation of this study was that both groups had baseline differences in age. However, the subjects were classified in pubertal and post-pubertal stages, which reduce the effect of age. Another limiting factor was the small number of subjects that prevented the division and analysis of groups according to sex.

CONCLUSIONS

Twelve weeks of HIIT were effective in promoting a decrease in BChE activity and, thus positive changes in the subjects’ cardiometabolic risk factors. This study strengthens the association of BChE with obesity and lipid metabolism in children and adolescents, and it shows that the reduction in BChE activity is simultaneous with reductions in WC and lipid profile as a direct result of HIIT.

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REFERENCES


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