

Effect of elevated physical activity on changes in body composition and subcutaneous fat distribution in boys aged 10 to 16 years: a longitudinal study

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ABSTRACT: The study is aimed at evaluation of the effect of regular physical activity on total and subcutaneous body fat and its distribution in boys aged 10 to 16 years. A three-year longitudinal study was carried out in order to monitor physical development in 237 boys from sports schools and regular schools in Warsaw, Poland. The boys were selected so that their rate of puberty changes was similar based on evaluation of voice and facial hair. The authors measured 5 skinfolds in the following sites: triceps, calf, subscapular, suprailiac, and abdominal skinfolds. The percentage fraction of total body fat in body mass was measured by means of Tanita TBF 300 electronic body composition analyser. A limb fat to trunk fat ratio (LF/TF) was also calculated in order to evaluate the type of distribution of subcutaneous fat in boys and monitor its changes as affected by regular high physical activity throughout puberty. Lower total body fat and subcutaneous fat in boys from sports schools was the effect of considerably higher physical activity. It was demonstrated that with some minimal values of total body fat and subcutaneous fat, physical activity did not cause a reduction in body fat. It was found that elevated physical activity in boys is conducive to development of a more limb-oriented (peripheral) fatness, which is more favourable to human health.

KEY WORDS: sports schools, skinfolds thickness, limb-oriented fatness, peripheral fatness, trunk fatness

Introduction

Physical activity is one of the most important lifestyle factors that determine human health status and biological growth. The differences in body build

are generally known, e.g. in the aspect of tissue components in physically active children and those with low physical activity (Malina 1979, Czezelewski and Raczyński 2006). The effect of regular exercise on changes in body tissue

components which causes a reduction in total body fat, increase in lean body mass, reduction in subcutaneous fat and changes in its distribution has been also demonstrated in studies of overweight and obese children and young people (Gutin et al. 2002, Meyer et al. 2006). Considerably fewer reports have monitored the effect of regular exercise on physical development in children and young people in the aspect of changes in body tissue composition in longitudinal studies (Courteix et al. 2001, Malina et al. 2004, Lewandowska et al. 2006, Medeková et al. 2007). The typical problem in this type of monitoring is selection of reference and control groups. The population of athletes usually include subjects with puberty changes that occur earlier compared to the control group. Therefore, the results that have demonstrated advanced growth in those physically active are due to the selection method rather than pure effect of physical activity (Malina et al. 2004, Lewandowska et al. 2006, Gil et al. 2010, Volver et al. 2010). It is known that the essential health effect is not only from total body fat but also from the type of fatness, connected with distribution of subcutaneous fatty tissue in different areas of human body (Szeklicki et al. 2000, Chrzanowska 1997). The central (trunk-based) type of fatness, typical of men, with particular excess body fat in abdominal areas significantly elevates the risk of cardiovascular system diseases and metabolic disorders connected with obesity (Bonora et al. 1996, Chrzanowska 1997). Even in a group of low risk (students from University School of Physical Education in Poznań, Poland), a positive correlation was found between WHR ratio and: blood pressure, level of triglycerides and the ratio of triglycerides

to HDL (Szeklicki et al. 2000). The relatively high fat in limbs compared to body trunk i.e. peripheral fatness, typical of children (Chrzanowska and Suder 2008) and young women (Suder and Gwardjak 2003) is connected with lower health risk, even with excessive total body fat (Chrzanowska 1997). One study found correlations of increased body blood and reduction in HDL fraction in children and young people aged 13 to 27 years in Danish population with increased ratio of subscapular to triceps skinfolds (Van Lenthe et al. 1998). A model of body fat distribution is significantly determined genetically (Malina et al. 1982, Bouchard 1997). However, there are the reports that have demonstrated the effect of environmental factors such as social status and lifestyles on distribution of fat (Bogin and Sullivan 1986). Furthermore, there are few publications that have evaluated the effect of physical activity on physical development of children and young people in the aspect of fat distribution with particular focus on children outside the risk group in longitudinal studies (Gutin and Owens 1999).

The aim of the present study was to evaluate the effect of regular high physical activity on total and subcutaneous body fat and its distribution in boys aged 10 to 16 years by means of comparison of these characteristics in children with similar rate of puberty changes from sports schools and non-sport schools in individual age groups.

Materials and Methods

The examinations were positively approved by the Senate's Research Bioethics Commission at the Józef Piłsudski University of Physical Education in Warsaw, Poland. The study participants were

informed about the goal of the study, methodology and procedures and the possibility of stopping the experiment at any moment. The subjects (their legal guardians) gave written consent to participate in the experiment.

In 2003–2005, the authors monitored physical development of boys from Warsaw's sports schools and non-sports schools, aged 10, 11, 13 and 14 years. For comparison purposes, the authors selected boys with similar rate of puberty changes based on the evaluation of voice and facial hair on a 3-degree scale (Lewandowska and Pastuszak 2011). Of 302 boys, the authors chose 237 students: 119 from sports schools in Warsaw and 118 subjects from regular schools.

In the sports schools, students participated in scheduled sports activities for 15 to 25 hours a week. In non-sport schools, the examinations focused only on those students who took part in compulsory physical education classes that ranged from 2 to 4 hours per week and declared additional recreational activity that did not exceed 4 hours a week.

The authors measured skinfold thickness in five sites: triceps, calf, subscapular, suprailiac and abdominal skinfolds using a calliper (Siber Hegner, Switzerland) (Lewandowska et al. 2011). Percentage fraction of total body fat in body mass was measured by means of Tanita TBF 300 electronic body composition analyser, Japan (Buśko and Lipińska 2012). Body height, mass and skinfold thickness was measured with the accuracy of 0.01 cm, 0.1 kg and 0.001 m, respectively. Maximum relative repeatability error for measurements of skinfolds expressed by the variability index ranges from 1.6% to 3.0% depending on the skinfold site, whereas fat analysis using BIA method yielded 0.3%.

The results obtained were used for calculation of the limb fat to trunk fat ratio (LF/TF) that represents subcutaneous tissue distribution:

$$\text{LF/TF} = \left[\frac{\text{skinfold above triceps} + \text{calf skinfold}}{\text{subscapular skinfold} + \text{suprailiac skinfold} + \text{abdominal skinfold}} \right] \cdot 100$$

In order to verify the results obtained during each measurement, the authors used ANOVA analysis of variance in the system with repeatable measurement. In order to compare the results between the two groups, the ANOVA/MANOVA analysis of variance was also used. The significance of differences between the means was evaluated using Tukey's test. A level of significance for the statistical analyses was set at $p < 0.05$. All the calculations were carried out by means of STATISTICA software (version 9.0 Stat-Soft, USA).

Results

During the three-year monitoring of tissue components in boys from sports schools and in control group, an insignificant decline in percentage of total body fat in body mass was found in both groups, except for those 14 years old. Total fatness was reduced in the control group by 15.8% and 3.3%, respectively, and by 13.3% and 6.6%, respectively, in the group of trained subjects within 3 years, with the exception of the boys from sports schools who exhibited an increase in body fat by 6.8% (Tables 1–4).

The changes in subcutaneous fatness in boys had dissimilar pattern. A reduction in the thickness of 5 skinfolds was observed at the level of 6% in the group of boys aged 10 and 11 and 8.2% in the trained subjects, whereas fatty layer rose in the untrained boys from 2.3%

Table 1. Characteristics of 10-year-old boys measured in 2003, 2004 and 2005

Characteristics		Boys from sports schools (n=25) Mean±SD	Δ	Controls (n=11) Mean±SD	Δ
Age (year)	Measurement 1	10.2±0.2		10.3±0.1	
	Measurement 2	11.2±0.2		11.3±0.2	
	Measurement 3	12.2±0.2		12.1±0.2	
Height (cm)	Measurement 1	143.6±4.9		145.0±5.6	
	Measurement 2	148.8±6.1	5.2	149.5±5.8	4.5
	Measurement 3	154.5±6.8	10.9	155.4±5.3	10.4
Weight (kg)	Measurement 1	34.1±4.7		36.0±8.6	
	Measurement 2	37.6±6.2	3.5	39.2±8.7	3.2
	Measurement 3	41.2±6.6	7.1	43.6±9.9	7.6
BMI (kg/m ²)	Measurement 1	16.5±1.6		17.0±3.0	
	Measurement 2	16.9±1.8	0.4	17.4±2.5	0.4
	Measurement 3	17.2±1.6	0.7	17.9±3.1	0.9
Fat mass (%)	Measurement 1	12.0±4.0		13.9±5.8	
	Measurement 2	11.3±3.7	-0.7	13.0±5.3	-0.9
	Measurement 3	10.4±3.4	-1.6	11.6±5.2	-2.2
Triceps skinfold (cm)	Measurement 1	1.02±0.34		1.21±0.49	
	Measurement 2	1.06±0.33	0.04	1.27±0.43	0.06
	Measurement 3	1.00±0.35	-0.02	1.20±0.45	-0.01
Subscapular skinfold (cm)	Measurement 1	0.72±0.26		0.96±0.60	
	Measurement 2	0.75±0.30	0.03	0.94±0.58	-0.02
	Measurement 3	0.64±0.20	-0.08	0.93±0.71	-0.03
Suprailiac skinfold (cm)	Measurement 1	0.91±0.50		1.16±0.64	
	Measurement 2	1.01±0.59	0.1	1.18±0.73	0.02
	Measurement 3	0.89±0.47	-0.02	1.26±0.79	0.10
Abdominal skinfold (cm)	Measurement 1	0.95±0.71		1.05±0.69	
	Measurement 2	1.00±0.73	0.05	1.17±0.84	0.12
	Measurement 3	0.87±0.60	-0.08	1.10±0.76	0.05
Calf skinfold (cm)	Measurement 1	1.11±0.36		1.27±0.61	
	Measurement 2	1.13±0.36	0.03	1.30±0.55	0.03
	Measurement 3	1.02±0.35	-0.09	1.31±0.67	0.04
Σ 5 skinfolds	Measurement 1	4.70±2.09		5.66±2.86	
	Measurement 2	4.96±2.17	0.26	5.87±3.02	0.21
	Measurement 3	4.42±1.72	-0.28	5.79±3.23	0.13

Measurement 1 was carried out in 2003, Measurement 2 – in 2004, Measurement 3 – in 2005;

Δ – difference between Measurement 1 and Measurement 2 or Measurement 1 and Measurement 3.

Table 2. Characteristics of 11-year-old boys measured in 2003, 2004 and 2005

Characteristics		Boys from sports schools (n=13) Mean±SD	Δ	Controls (n=37) Mean±SD	Δ
Age (year)	Measurement 1	10.7±0.1		10.8±0.2	
	Measurement 2	11.7±0.1		11.8±0.3	
	Measurement 3	12.7±0.1		12.8±0.2	
Height (cm)	Measurement 1	147.9±6.2		148.2±6.1	
	Measurement 2	152.6±6.5	4.7	154.1±7.2	5.9
	Measurement 3	158.9±7.8	11.0	161.1±8.5	12.9
Weight (kg)	Measurement 1	37.8±6.0		38.4±7.5	
	Measurement 2	40.6±6.0	2.1	43.3±8.6	4.9
	Measurement 3	46.4±7.6	8.6	48.6±9.7	5.3
BMI (kg/m ²)	Measurement 1	17.2±1.9		17.4±2.9	
	Measurement 2	17.4±1.7	0.2	18.1±2.9	0.7
	Measurement 3	18.3±1.9	1.1	18.6±3.0	1.2
Fat mass (%)	Measurement 1	12.2±3.2		14.2±5.3	
	Measurement 2	11.3±3.5	-0.9	13.8±6.0	-0.4
	Measurement 3	11.4±3.2	-0.8	12.4±6.1	-1.8
Triceps skinfold (cm)	Measurement 1	1.10±0.42		1.27±0.53	
	Measurement 2	1.08±0.42	-0.02	1.39±0.56	0.12
	Measurement 3	1.03±0.36	-0.07	1.38±0.54	0.11
Subscapular skinfold (cm)	Measurement 1	0.81±0.38		0.96±0.68	
	Measurement 2	0.80±0.28	-0.01	1.06±0.67	0.10
	Measurement 3	0.80±0.30	-0.01	1.11±0.67	0.15
Suprailiac skinfold (cm)	Measurement 1	1.06±0.64		1.27±0.80	
	Measurement 2	1.04±0.46	-0.20	1.36±0.74	0.09
	Measurement 3	0.93±0.43	-0.13	1.40±0.77	0.13
Abdominal skinfold (cm)	Measurement 1	1.01±0.67		1.22±0.74	
	Measurement 2	1.04±0.67	0.03	1.51±0.91	0.29
	Measurement 3	0.94±0.57	-0.07	1.49±0.93	0.27
Calf skinfold (CM)	Measurement 1	1.24±0.65		1.46±0.82	
	Measurement 2	1.15±0.45	-0.09	1.50±0.65	0.04
	Measurement 3	1.09±0.46	-0.15	1.60±0.81	0.14
Σ 5 skinfolds	Measurement 1	5.22±2.68		6.18±3.33	
	Measurement 2	5.12±2.18	-0.10	6.82±3.25	0.64
	Measurement 3	4.79±1.93	-0.43	6.98±3.52	0.80

Measurement 1 was carried out in 2003, Measurement 2 – in 2004, Measurement 3 – in 2005;

Δ – difference between Measurement 1 and Measurement 2 or Measurement 1 and Measurement 3.

Table 3. Characteristics of 13-year-old boys measured in 2003, 2004 and 2005

Characteristics		Boys from sports schools (n=12) Mean±SD	Δ	Controls (n=37) Mean±SD	Δ
Age (year)	Measurement 1	13.3±0.2		13.1±0.2	
	Measurement 2	14.3±0.1		14.2±0.2	
	Measurement 3	15.3±0.1		15.2±0.2	
Height (cm)	Measurement 1	162.2±8.2		158.8±8.5	
	Measurement 2	169.5±9.7	7.3	166.2±8.7	7.4
	Measurement 3	176.1±8.6	13.9	171.9±7.4	13.1
Weight (kg)	Measurement 1	48.4±7.3		49.3±11.8	
	Measurement 2	55.8±8.7	7.4	56.1±12.3	6.8
	Measurement 3	62.8±8.1	14.4	61.6±13.2	12.3
BMI (kg/m ²)	Measurement 1	18.3±2.1		19.4±3.9	
	Measurement 2	19.4±2.1	1.1	20.2±3.6	0.8
	Measurement 3	20.2±1.9	1.9	20.9±4.2	1.5
Fat mass (%)	Measurement 1	10.3±3.7		13.9±8.1	
	Measurement 2	9.5±4.3	-0.8	12.7±6.7	1.2
	Measurement 3	9.6±4.3	-0.7	12.1±6.8	-1.8
Triceps skinfold (cm)	Measurement 1	1.01±0.33		1.42±0.73	
	Measurement 2	0.96±0.38	-0.05	1.33±0.67	-0.09
	Measurement 3	0.95±0.38	-0.06	1.21±0.65	-0.21
Subscapular skinfold (cm)	Measurement 1	0.76±0.35		1.26±0.81	
	Measurement 2	0.83±0.40	0.07	1.28±0.84	0.02
	Measurement 3	0.87±0.27	0.11	1.11±0.67	-0.15
Suprailiac skinfold (cm)	Measurement 1	1.08±0.49		1.64±1.06	
	Measurement 2	1.02±0.54	0.06	1.52±0.98	-0.12
	Measurement 3	1.15±0.62	0.07	1.55±0.98	-0.09
Abdominal skinfold (cm)	Measurement 1	1.06±0.50		1.78±1.19	
	Measurement 2	1.01±0.47	-0.05	1.61±1.13	-0.17
	Measurement 3	1.08±0.51	-0.02	1.60±1.02	-0.18
Calf skinfold	Measurement 1	1.33±0.44		1.82±1.08	
	Measurement 2	1.15±0.48	-0.18	1.52±0.87	-0.30
	Measurement 3	1.12±0.55	-0.21	1.41±0.95	-0.41
Σ 5 skinfolds	Measurement 1	5.24±1.92		7.92±4.59	
	Measurement 2	4.96±2.14	-0.28	7.26±4.28	-0.66
	Measurement 3	5.16±2.22	-0.08	6.99±4.23	-0.93

Measurement 1 was carried out in 2003, Measurement 2 – in 2004, Measurement 3 – in 2005;

Δ – difference between Measurement 1 and Measurement 2 or Measurement 1 and Measurement 3.

Table 4. Characteristics of 14-year-old boys measured in 2003, 2004 and 2005

Characteristics		Boys from sports schools (n=69) Mean±SD	Δ	Controls (n=33) Mean±SD	Δ
Age (year)	Measurement 1	13.9±0.3		13.9±0.2	
	Measurement 2	14.8±0.3		15.0±0.2	
	Measurement 3	15.8±0.3		16.0±0.3	
Height (cm)	Measurement 1	170.1±9.2		169.6±8.1	
	Measurement 2	175.5±8.3	5.4	176.2±7.3	6.6
	Measurement 3	179.1±7.2	9.0	179.1±6.8	9.5
Weight (kg)	Measurement 1	55.3±10.2		58.7±12.3	
	Measurement 2	62.1±10.8	6.8	64.9±12.5	6.2
	Measurement 3	66.3±9.2	11.0	69.4±14.1	10.7
BMI (kg/m ²)	Measurement 1	19.0±2.4		20.3±3.4	
	Measurement 2	20.1±2.8	1.1	20.8±3.3	0.5
	Measurement 3	20.6±2.6	1.6	21.5±3.6	1.2
Fat mass (%)	Measurement 1	8.8±3.6		12.2±6.4	
	Measurement 2	9.5±4.7	-0.7	11.4±6.3	-0.8
	Measurement 3	9.4±3.9	0.6	11.8±6.6	-0.4
Triceps skinfold (cm)	Measurement 1	0.83±0.26*		1.33±0.71*	
	Measurement 2	0.88±0.34	0.05	1.20±0.57	-0.13
	Measurement 3	0.83±0.26	0	1.14±0.54	-0.19
Subscapular skinfold (cm)	Measurement 1	0.72±0.21*		1.11±0.75*	
	Measurement 2	0.81±0.33	0.09	1.08±0.55	-0.03
	Measurement 3	0.84±0.25	0.12	1.22±0.75	0.11
Suprailiac skinfold (cm)	Measurement 1	0.89±0.41*		1.46±0.78*	
	Measurement 2	1.01±0.63	0.12	1.53±1.02	0.07
	Measurement 3	0.98±0.42*	0.09	1.51±0.98*	0.05
Abdominal skinfold (cm)	Measurement 1	0.94±0.44*		1.57±1.02*	
	Measurement 2	1.04±0.54	0.10	1.58±0.97	0.01
	Measurement 3	1.03±0.51*	0.09	1.64±1.15*	0.07
Calf skinfold (cm)	Measurement 1	1.01±0.28*		1.52±0.70*	
	Measurement 2	1.01±0.44	0	1.39±0.75	-0.13
	Measurement 3	0.89±0.29	-0.12	1.30±0.76	-0.22
Σ 5 skinfolds	Measurement 1	4.39±1.45*		6.99±3.71*	
	Measurement 2	4.75±2.11	0.36	6.79±3.67	-0.20
	Measurement 3	4.58±1.56*	0.19	6.81±3.97*	-0.18

Measurement 1 was carried out in 2003, Measurement 2 – in 2004, Measurement 3 – in 2005;
 Δ – difference between Measurement 1 and Measurement 2 or Measurement 1 and Measurement 3.
 * – indicates statistically significant differences from the groups, $p < 0.05$

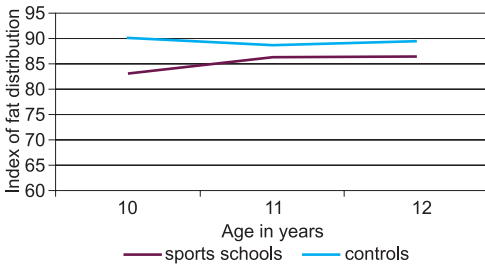


Fig. 1. Changes in limb fat to trunk fat ratio in the group of boys aged 10 years

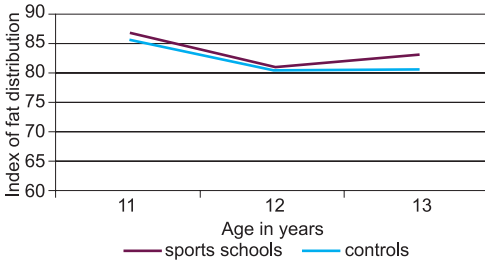


Fig. 2. Changes in limb fat to trunk fat ratio in the group of boys aged 11 years

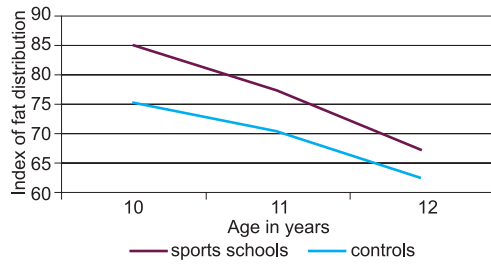


Fig. 3. Changes in limb fat to trunk fat ratio in the group of boys aged 13 years

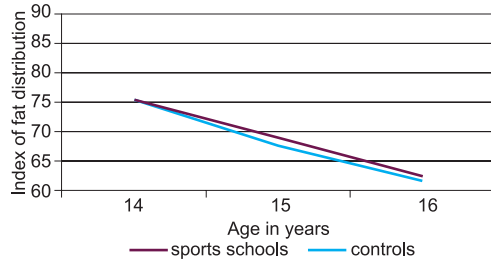


Fig. 4. Changes in limb fat to trunk fat ratio in the group of boys aged 14 years

to 12.9% (Tables 1–2). In older 13-year-old and 14-year-old cohorts, a decrease in subcutaneous fatness by 11.7% and 2.6%, respectively, was observed in the control group. In the trained 13-year-old boys, the change amounted to –1.5%, whereas in the 14-year old from sports school this value was 4.3% (Tables 3–4). Significant differences between the groups were observed in boys aged 14 years old for individual skinfolds and their sums.

Fig. 1–4 presented value of limb fat to trunk fat (LF/TF) ratio that represents distribution of subcutaneous fatness, i.e. the percentage ratio of the sum of skinfold thickness measurements over triceps and calf to the sum of body trunk skinfolds (subscapular, abdominal and suprailiac skinfolds). Greater values of this ratio are typical of peripheral type of fat distribution. The level of LF/TF ratio in

boys from both types of schools declined with age by 25–30% in both groups of boys aged 13 to 14 years (Figures 3–4). In younger categories, regardless of the school type, the value of the ratio ranged from 80 to 90% (Figures 1–2). The limb fat to trunk fat ratio exhibited similar tendencies in boys from the compared schools. However, in the category of boys aged 13 years, the difference at the level of 10% of LF/TF value confirms a more peripheral type of fat distribution in trained subjects compared to the control group (Figure 3).

No differences were found between mean values of body height throughout three years of the observations. The increase in this parameter in the group of trained subjects and the control group was at the level of 8% (Tables 1–3), whereas in the oldest age cohort it amounted to ca. 5% (Table 4).

Discussion

The examinations of trained children and young people demonstrated that selection in a variety of training departments (Malina et al. 2004, Gil et al. 2010) or sports schools (Lewandowska et al. 2006, Lewandowska and Pastuszak 2011) is usually focused on boys with early rate of puberty changes due to better results obtained by them in qualification tests. The examination of the effect of increased physical activity on physical development and body build in these selected groups is difficult because the differences usually reflect the selection procedures rather than the effect of physical activity. In our study, the referential and control groups were selected with consideration of similar rate of puberty changes in order to exclude the effect of sport selection on somatic effects of increased physical activity. This allowed for elimination of the above misleading conclusions. The boys with similar rate of puberty changes did not differ in terms of basic somatic traits, fatness and body build in terms of weight-height ratio. However, no significant differences in subcutaneous and total body fat were found in our three-year study in physically active boys compared to the control group, except for those 14 year old. The percentage of total fat in body mass in boys from both types of school showed a trend that decreased with age, which is typical of developmental patterns in this gender (Tanner 1962, Chrzanowska and Suder 2008). In the puberty period, the boys exhibited a decreasing ratio of total fat in body mass (increase in lean body mass) and a rise in the sum of 5 skinfolds (relative reduction in fatty layer, particularly in the group of physically active subjects). However, these differ-

ences were not statistically significant. It is also remarkable that with certain minimal values of total and subcutaneous fatness, even intensive physical activity does not cause any reduction in body fat, which is genetically determined (Malina et al. 2004).

Our results are inconsistent with the results of four-year observations of children in Slovakia which demonstrated that trained boys were higher, heavier and exhibited less fat than the untrained peers (Medeková et al. 2007).

The index of relative fatness in limbs compared to body trunk had similar values and exhibited similar developmental patterns in both groups of boys compared in the study. The changes observed in the LF/TF ratio in boys aged 13 and 14 years suggest tendencies of transition of the type of fatness in male subjects from peripheral (limb-oriented) type into the central (trunk-oriented) fatness. This corresponds to the results obtained by Chrzanowska and Suder (2008). Only the category of 13-year-old boys had more peripheral type of fatness (more favourable for human health) in physically active boys (Gutin and Owens 1999). However, the difference of 10% between the compared groups was not statistically significant. The study by Meyer et al. (2006) demonstrated that a regular six-week exercise routine caused a reduction in subcutaneous fatness and WHR ratio and a decline in triglyceride levels in obese teenagers of both genders aged 11 to 16. Similar increments in subcutaneous fatty tissue and overall fatness were observed during three years in both groups of boys analysed in the present study. It is remarkable that with certain minimum values of total fat (8.8%) and subcutaneous fat (sum of 5 skinfolds: 4.39 mm)

found in the group of trained boys aged 14 years, even intensive physical activity (25 hours a week) does not cause a reduction in fatness as its level is genetically determined. Increased physical activity did not affect the processes of growth in the group of boys from sports schools. Similar results were obtained in a three-year study of female gymnasts, where 15 to 22-hour weekly training regime did not disturb the processes of growth (Courteix et al. 2001).

Conclusions

Lower total body fat and subcutaneous fat in boys from sports schools is the effect of considerably higher physical activity.

With some minimal values of total body fat and subcutaneous fat, physical activity does not cause a reduction in body fat.

More intensive physical activity in boys is conducive to development of a more limb-oriented (peripheral) fatness, which is more favourable to human health.

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Author Contribution

AP wrote the article, collected the data, interpretation of results; JL provided logistical support during data collection and reviewed the manuscript, statistical analysis and interpretation of the data was executed by KB; JCh edited the manuscript.

Conflict of interest

The Authors declare that there is no conflict of interests regarding the publication of this article.

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