Acute phase proteins disturbances indicate uncompensated obesity declining physical fitness

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Abstract. – OBJECTIVE: The correlation between physical fitness and health indicators still requires a research aimed at improving the knowledge about physical fitness and the impact of obesity on the health status in children and adolescents. The aim of this study is an analysis of the results of the EUROFIT battery tests in relation to routine laboratory parameters and the inflammation markers.

PATIENTS AND METHODS: In the group of 123 Polish adolescents the routine parameters of lipid metabolism and acute phase proteins were investigated, and compared with EUROFIT motor fitness tests results, expressed as percentiles of the results achieved by healthy Polish population.

RESULTS: Most of the EUROFIT tests battery were performed by overweight adolescent girls on an insufficient level. Children who were described by parameters indicating more advanced obesity performed the EUROFIT tests worse. There was showed a negative correlation between the concentration of HDL cholesterol and the long jump (rho=-0.304) as well as with the speed of limb movement (rho=-0.277). There was a positive correlation between the speed of limb movement and the concentration of triglycerides (rho=0.335), LDL cholesterol (rho=0.305) and the percentage of the A4 (rho=0.239).

CONCLUSIONS: Disturbed lipid parameters, as well as altered glycosylation profiles of acute phase proteins, were observed in all overweight children, and the intensity of alterations correlated with worse fitness.

Key Words:
Acute phase proteins, Physical fitness, Obesity, Adolescent, EUROFIT.

Introduction

Overweight and obesity are among the pressing challenges facing public health. This problem particularly concerns the population of children and adolescents[1-3], who are at risk of obesity and disability in the future. This is the reason why provisions related to the search for correlates and determinants of obesity for various age and social groups are included in many documents and strategies on the health policy worldwide[4], in Europe[5,6], and Poland[7,8]. Rising risk of obesity can be observed in both the developed and the developing world. These trends show that 1 out of 3 inhabitants of the world is overweight[9,10], and that 1 out of 5 children suffers from overweight, including obesity[11]. According to the World Health Organization, there was a huge worldwide prevalence of childhood overweight and obesity starting from the level of 4.2% in 1990, reaching the level of 6.7% in 2010, with a prognosis of 9.1% in 2020[12].

The epidemiology of obesity in Poland, especially in children and adolescents, also raises increasing concern. Population studies by Jodkowska et al[13] of children in Poland aged 13-15 show a 2% increase in the prevalence of overweight and obesity in the years 1995-2005. The prevalence of overweight and obesity was 12.5% and 1.9%, respectively in 1995. No significant sex differences were found, either in overweight or obesity.

The correlation between physical fitness and health indicators in children and adolescents still requires a lot of research aimed at improving the knowledge about physical fitness and the impact of obesity on the health status in children and adolescents.

Many authors believe that there are clear genetic factors that predetermine the occurrence of overweight or obesity[14], and other health problems[15] in people. Knowledge of the family history of overweight or obesity and immune system disorders is of much help. Such disorders

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may lead, for instance, to changes in the concentrations of inflammation markers, including acute-phase proteins. Home environment often determines a healthy or an unhealthy lifestyle of a teenager, which is why the information about the family environment helps us get a deeper insight into the health status and predispositions to the disease, such as overweight or obesity. However, regardless of the genetic background obesity is often determined by lifestyle.

There are many tests and scales for the assessment of physical fitness. The Eurofit Physical Fitness Test Battery (EUROFIT) is a test that is frequently used for the assessment of physical fitness elements related to health. Although the test is time-consuming, it is selected by many authors as it checks a number of physical fitness elements, such as balance, speed, flexibility, strength (explosive, static, functional), agility, cardiorespiratory endurance. Scoring tables for this test in the form of percentile charts have been developed in Poland. These charts allow a detailed assessment of a given sample regardless of age or sex[16].

The aim of this study is a re-analysis of the results of the EUROFIT battery tests, presented in previous publications on the study group[17] but in relation to routine laboratory parameters and inflammation markers as indicated by acute phase proteins.

Patients and Methods

The study enrolled 123 teenagers aged 11-18. The study group included 62 boys and 61 girls who were overweight (BMI between 25 and 30) or obese (BMI above 30). The mean body weight was 89.32 ± 17.69, the mean height was 168.6 ± 9.82, the mean BMI was 31.27 ± 4.38.

For each adolescent BMI was calculated according to the formula: (weight/height^2; kg/m^2). Participants were considered overweight or obese based on age-specific BMI curves when their BMI was more than or equal to the international cutoff point corresponding to the curve that passes through the BMI curve of either 25 or 30, respectively, at the appropriate age[18]. Weight was assessed to the nearest 0.1 kg using a certified electronic scale (Tanita electronic scale BWB-800, Tanita Europe BV, Hoogoorddreef 56e, 1101BE, Amsterdam, The Netherlands), with the subject wearing sports clothing and no shoes. Height to the nearest 0.01 m was measured using a stadiometer (Holtain Ltd., Crymych, Pembs, UK). Physical examination was carried out to exclude all symptoms of on-going infection or inflammation of any origin. The study was carried out in Ciechocinek in the years 2010-2012.

Physical fitness was measured using the EUROFIT test which consists of: flamingo balance (FLB – balance), plate tapping (PLT – coordination and speed), sit-and-reach (SAR- flexibility), standing broad jump (SBJ- explosive strength), handgrip (HGR – static strength), sit-ups (SUP – trunk strength and endurance), bent-arm hang (BAH – upper body strength and endurance) and shuttle run 10×5 m (SHR – speed and agility), 20 m endurance shuttle run (ESR – cardio-respiratory endurance)[19]. The results are presented in the form of a percentile chart based on age and sex and developed for Polish children and adolescents[16].

Blood samples were collected once from all participants. Serum was separated in a routine way and frozen until investigation. The concentration of acute phase proteins: alpha1-acid glycoprotein (AGP); and alpha1-antichymotrypsin (ACT) was measured by immunoelectrophoresis according to Laurell[20]. Antibodies and standard solutions came from DakoCytomation (Dako Polska, Gdynia, PL). Additionally, the glycosylation profiles of AGP and ACT were analyzed by crossed-affinity immunoelectrophoresis with Concanavalin A[20]. Reference values for acute-phase proteins in children were established earlier in the same laboratory, using the same methods. Normal serum values for AGP and ACT have been reported to be 800±100 mg/L and 400±50 mg/L, respectively[21].

Statistical Analysis

The description of the investigated variables was performed using the mean with a standard deviation (demographic data, protein parameters) or medians and quartiles (for the EUROFIT test) respectively. The comparison of the investigated subgroups was performed using the U Mann-Whitney test for two groups, and Dunn's test as post-hoc if necessary, chi^2 test for distribution of the data, and the correlations were calculated using the Spearman test. The Statistica 10.0. software was used for this purpose. $p < 0.05$ was considered statistically significant.

Results

Mean values with standard deviations as well as reference values for all investigated parameters, for the whole investigated group are summarized in Table I.

The majority of the lipid parameters were within the reference values. In some subject, only LDL values significantly exceeded the reference values. If the study group was divided into subjects with the percentage of HDL cholesterol above 30 and those who did not exceed this level (n=24 vs. n=99), it could be shown that mainly girls (21 girls vs. 3 boys) constituted the subgroup with the normal percentage of HDL. There was a statistically significant difference in the LDL concentration in favor of the group with the normal HDL percentage (2.3±0.5 vs. 28.2±10.4; F=506.0 p=0.000). In this subgroup the total AGP (574±189 vs. 1121±541; F= 8.2 p=0.005) and ACT (285 ± 48 vs. 491±202; F=17.9 p=0.000) concentration and the percentage of the W2 AGP variant were significantly lower.

Some elements of EUROFIT test battery (SAR and HGR) were performed well by a relatively large part of the children. On the contrary, the majority of tests were performed very badly: flamingo balance (FLB), standing broad jump (SBJ), sit-ups (SUP), bent-arm hang (BAH) shuttle run (SHR) or endurance run (ESR). Exact results are presented in Table II.

When the children were classified according to percentiles, as presented in Table II, and the biochemical parameters, as well as acute phase proteins, were analyzed by Kruskal-Wallis ANOVA (the results were regarded as significant if p<0.05), following differences were found. For BAH: AGP-W3% – H=5.40; p=0.014; similar though not significant difference for ACT-A3%; higher LDL was observed for children below 50 percentiles. For SAR: HDL-cholesterol% and AGP-W3%: worse percentiles for children with lower HDL% or higher W3; not significant. For ESR: triglycerides higher in children below 50 percentiles, not significant.

When children were classified into two groups only, "<10 percentile" and "higher" for the worst tests (FLB, PLT, SBJ, SUP, BAH, ESR, SHR), or ">90 percentile" and "less" for the best tests (SAR and HGR), following differences could be found in U Mann-Whitney test: PLT: triglycerides (Z=2.34, p=0.019); LDL (Z=2.65, p=0.008); HDL% (Z=-2.06, p=0.040); ACT-A4% (Z=2.36, p=0.018) - with worst results observed for worse performers; SBJ: HDL (Z=2.06, p=0.040) – with worst results observed for worse performers; BAH: AGP-W3% (Z=2.17, p=0.030), higher W3% was observed for worse performers.

Next, the numbers of children divided according to parameters describing obesity were compared to classification according to percentiles. Using the chi^2 test, the distribution was analyzed (the results were regarded as significant if *p*<0.05), and the results are shown in Table III.

Table I. Results of the investigated laboratory parameters for the entire study group (n=123).

Parameter	*Mean value ± SD*	*Reference values*	*Difference girls vs. boys U Mann-Whitney test*
GLU mmol/l	4.2±0.5	3.9-6.4	ns
CHOL mmol/l	4.2±0.8	< 5.2	ns
LDL mmol/l	23.8±13.6	< 3.5	ns
HDL mmol/l	♂ 2.8±0.9	♂ 0.9-1.8	**Z=2.45 *p*=0.014**
	♀ 2.2±0.9	♀ 1.0-2.1	
HDL%	♂ 0.3-35.7	> 30	**chi^2=5.17 *p*=0.023**
	♀ 0.5-44.5		
TG mmol/l	1.1±0.4	0.55-2.3	ns
AGP mg/l	1037±475	700-900	ns
W0%	41±9	43	ns
W1%	43±6	45	ns
W2%	13±7	12	ns
W3%	0-24	0	ns
ACT mg/l	419±172	350-450	ns
A1%	26 ±12	25	ns
A2%	32±8	24	ns
A3%	23±9	26	ns
A4%	14±10	25	ns
A5%	0-18	0	ns

Table II. Percentile division of EUROFIT tests; numbers for each category are provided (n=123).

EUROFIT tests results in percentiles		Difference girls vs. boys, chi^2 test	<10	10-25	25-50	50-75	75-90	>90
Flamingo balance	♀ n=61		47	5	8	0	1	0
(FLB)	♂ n=62	ns	45	8	4	0	4	1
Plate tapping	♀ n=61		25	9	12	6	6	3
(PLT)	♂ n=58	ns	22	13	11	7	3	2
Standing broad	♀ n=51		35	16	7	3	0	0
jump (SBJ)	♂ n=62	ns	44	11	6	1	0	0
Hand grip (HGR)	♀ n=58		3	6	15	19	11	6
	♂ n=60	ns	11	11	10	11	11	4
Sit-ups (SUP)	♀ n=61		39	10	6	4	0	2
	♂ n=62	ns	33	13	12	3	1	0
Bent-arm hang	♀ n=61		54	4	2	1	0	0
(BAH)	♂ n=62	ns	48	7	4	3	0	0
Endurance run	♀ n=57	chi^2=18.17;	25	9	6	7	10	4
(ESR)	♂ n=62	p=0.002	33	8	5	16	0	0
Shuttle run (SHR)	♀ n=61	chi^2=11.37;	35	19	5	0	0	2
	♂ n=58	p=0.044	42	11	1	2	2	0
Sit and reach (SAR)	♀ n=61	ns	6	2	1	9	14	29
	♂ n=62		9	3	4	11	11	24

Table III. Uneven distribution of the EUROFIT tests results and demographic parameters.

Children classified according to	Tests with uneven distribution		
BMI: 25-30 or >30	SUP: chi^2=11.15, p=0.048	ESR: chi^2=14.03, p=0.015	SAR: chi^2=10.81, p=0.055
WHR: ≤1 or >1			SAR: chi^2=13.28, p=0.021
HDL%: <30 or ≥30	SUP: chi^2=13.95, p=0.016		SAR: chi^2=10.98, p=0.050

Children described by parameters indicating more advanced obesity performed the EUROFIT tests worse. When the classification according to BMI was analyzed, 29 of 49 children with BMI lower than 30 were classified as >90 percentile in SAR test.

The distribution of children according to HDL% showed that more boys had values below 30% (the difference girls vs. boys: chi^2=5.17; p=0.002). Thus, the difference observed for the tests SUP and SAR in Table III may be attributed to differences between boys and girls, as observed for the whole group (Table II).

Next, correlations between the percentiles achieved in EUROFIT tests and biochemical parameters, as well as acute phase proteins were analyzed, using non-parametric Spearman's test. The results were regarded as significant if p<0.05.

The analysis of balance, EUROFIT- FLB, showed that the majority of children failed to perform this test. At the same time there was a positive, significant correlation between the proper execution of this test and the percentage of HDL cholesterol (rho=0.309). The majority of children also performed the PLT test badly, and fol-

Table IV. Results of the study of correlations (rho, Spearman test) between lipid parameters and the results for acute phase proteins. Only statistically significant correlations are shown (p<0.05).

	GLU	Total CHOL	TG	LDL	HDL	HDL%
AGP mg/l	-0.295		0.484	0.511	0.389	-0.540
W0%						0.305
W1%						
W2%						
W3%						
ACT mg/l			0.443	0.478	0.312	-0.530
A1%						
A2%						
A3%						
A4%			0.379	0.349		
A5%			-0.378	-0.383	-0.420	0.499

lowing statistically significant correlations could be found: with the concentration of triglycerides (rho=0.335); LDL cholesterol (rho=0.305), a negative correlation with HDL% (rho=-0.277), and a positive with the percentage of ACT-A4 variant (rho=0.239).

The analysis of the long jump showed a negative correlation with the concentration of HDL cholesterol (rho=-0.304). No correlations with the lipid parameters in the other EUROFIT tests have been shown.

Finally, we investigated correlations between the lipid parameters and the concentrations of acute phase proteins. The results are found in Table IV.

Statistically, significant correlations were shown primarily for the total concentrations of acute phase proteins. It was also shown that glycosylation variants demonstrating the presence of acute inflammation correlated positively with those lipid parameters that indicate disorders, and negatively with the percentage of HDL cholesterol.

When a division by the percentile of the endurance test was applied, it was demonstrated that the total concentration of AGP and the distribution of differently glycosylated variants of this protein showed more abnormalities in children with inferior scores for endurance. However, these differences were not statistically significant.

Discussion

As could be expected, greater severity of lipid metabolism disorders translated into more altered parameters indicating the severity of inflammation and more impaired physical fitness.

In the investigated group of obese children/adolescents, lipid disorders were related primarily to LDL cholesterol. It was demonstrated that this parameter correlated with increased concentrations and altered glycosylation of acute phase proteins, thus indicating liver dysfunction. This parameter can be regarded as an exceptionally unfavorable predictive exponent, according to the data found in the literature, even if the total concentration of cholesterol, triglycerides and glucose was normal in the subjects[17]. It is also worth noting that this parameter seems to indicate poor eating habits that are most frequently the cause of obesity in families.

In the same study, it was also pointed out that obesity can have more negative effects on boys[17]. The authors demonstrated a similar trend in the previous publication[17], which was confirmed by these results: in the group of obese adolescents, there were definitely more girls with a normal percentage of HDL cholesterol (and therefore at a significantly lower risk of obesity-related complications). It is also worth noting that the percentage of HDL cholesterol correlated with those results of the EUROFIT battery tests, which showed inferior physical fitness of the subjects. It could also be observed that children who performed worse in the ESR test had higher (though not significantly) LDL values.

The diminished results of the SAR or SUP test may be attributed to the visceral obesity, and were combined with worse BMI, WHR and HDL% results. Thus, not only the net percentage of the adipose tissue, nor BMI itself constitute the risk of deteriorating physical fitness, but the distribution of adipose tissue and the male sex.

It was also confirmed by the presented results that the declining physical fitness was combined with those parameters of acute phase proteins which indicate more intensive inflammatory process. It is known from previous studies[17,22] that adipose tissue may be responsible for increased production of interleukin-6, altering the glycosylation profiles of the investigated proteins towards variants more reactive with concanavalin A (less branched), appearing during acute inflammatory states. Acute phase reactants may also be regarded as “danger signal”, even as a predictor of death risk[23].

Cyclical international Health Behaviour in School-Aged Children research conducted in Poland allows tracking overweight and obesity trends in school-aged children, as well as selected determinants of this phenomenon. In the analysis of the research Mazur et al[24] show that in the years 2006-2010 the percentage of overweight students increased from 17.7% to 22.0% among boys and from 7.9% to 13.4% among girls. Body weight increase was not reported only in the group with proper habits. However, what is of concern is the reduction in the percentage of adolescents classified into this group. Such epidemiological indicators negatively differentiate us from other European Union countries, as we are leaders when it comes to the rate of the increase in the percentage of children with overweight and obesity[25].

Conclusions

1. Disturbed lipid parameters, as well as altered glycosylation profiles of acute phase proteins, were observed in all overweight children, and the intensity of alterations correlated with worse fitness.

2. Especially following tests from EUROFIT battery: SUP, BAH, SHR, ESR were performed badly by the investigated children. This points out to poor cardiorespiratory endurance, at least in part attributed to abdominal (visceral) obesity, being the most critical risk factor of the possible metabolic complications of obesity.

Competing interest

The study was conducted under a research grant of the Ministry of Science and Higher Education KBN N N404269639.

Conflicts of interest

The authors declare no conflicts of interest.

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