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Maximal oxygen uptake is associated with allele -202 A of insulin-like growth factor binding protein-3 (*IGFBP3*) promoter polymorphism and $(CA)_n$ tandem repeats of insulin-like growth factor *IGF1* in Caucasians from Poland

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Physical fitness is a trait determined by multiple genes, and its genetic basis is modified by numerous environmental factors. The present study examines the effects of the $(CA)_n$ tandem repeats polymorphism in *IGFI* gene and SNP *Alw21I* restriction site -202 A>C polymorphism in *IGF1BP3* on VO_2max — a physiological index of aerobic capacity of high heritability. The study sample consisted of 239 (154 male and 85 female) students of the University School of Physical Education in Poznań and athletes practicing various sports, including members of the Polish national team. An association was found between -202 A/C polymorphism of *IGFBP3* gene with VO_2max in men. Higher VO_2max values were attained by men with CC genotype, especially male athletes practicing endurance sports and sports featuring energy metabolism of aerobic/anaerobic character. A statistically significant influence of allele 188 and genotype 188/188 of tandem repeats $(CA)_n$ polymorphism of *IGF1* gene on VO_2max was found in women. Also, lower values of maximal oxygen uptake were noted in individuals with allele 186 or genotype 186/186, and higher VO_2max values in athletes with allele 194.

Key words: *IGF1*, *IGFBP3*, athletic performance, genetic polymorphism, energy efficiency

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INTRODUCTION

IGF-1 is a protein with multiple physiological functions. It belongs to the family of growth hormones, and acts in an endo-, para- and autocrine fashion (D'Ercole *et al.,* 1984; Stewart & Rotwein, 1996). Its molecular structure resembles proinsulin. IGF-1 consists of 70 amino acids in a single chain. It is produced in the liver and is a mediator of the activity of growth hormone (GH). Circulating IGF-1, produced as an endocrine hormone affected by GH, is responsible for correct growth and development. IGF-1 produced in target tissues is independent of GH, and functions as a growth factor in these tissues (Hameed *et al.,* 2002; Jernström *et al.,* 2001).

Epidemiological studies point to a large variability of IGF-1 level in the blood serum of healthy individuals and between ethnic groups, regardless of GH level (Platz *et al.,* 1999). IGF-1 phenotype is a complex hereditary trait affected by many genetic determinants, some of which may depend on a growth hormone (Rosen *et al.,* 1998; Gronek *et al.*, 2005). The contribution of the genetic component to IGF-1 blood level was estimated at 38–63% by various research teams in studies of adult twins (Cheng *et al.,* 2005; Harrela *et al.,* 1996; Hong *et al.,* 1996; Kao *et al.,* 1994).

The level of circulating IGF-1 decreases with age, and it might be responsible for the decline of body mass and muscle strength. Studies on animals revealed an influence of IGF-1 on the activation of myosatellite cells. A more significant role is attributed to the isoforms produced in muscle tissue (Devaney, 2007).

IGF1 gene consists of 5 exons. The first two out of them form the untranslated region with signal peptides. Exon 3 is the remaining sequence for the signaling protein and a part of domain B, while exon 4 is a part of domains B, C, A and D (Juul, 2003). IGF1 has two promoters at the 5' ends of exon 1 and 2. The transcripts starting from exon 2 are GH dependant and are produced in the liver, whereas transcripts from exon 1 are produced outside the liver, and are subject to alternative splicing. It results in three different peptides E with a mutual N-terminus sequence but different C-terminuses: IGF-1Ea, IGF-1Eb, IGF-1Ec (MGF). The overexpression of the first of these isoforms in murine muscles leads to hypertrophy and protects muscles against mass loss. Together with the third isoform, they are expressed in muscles following mechanical stimulation, i.e. physical activity (Hameed *et al.,* 2002).

Some cross-sectional studies showed the mean levels of circulating IGF-1 to be positively correlated with physical fitness or intensive physical activity (Juul, 2003; Eliakim *et al.,* 1996; Poehlman & Copeland, 1990; Lukanova *et al.,* 2001; Ambrosio *et al.,* 1996; Kelly *et al.,* 1990). Other authors found no correlations between IGF-1 level and physical activity and aerobic fitness either in young or elderly subjects (Jørgensen *et al.,* 1998; Kiel *et al.,* 1998). The results of studies on the effects of training on IGF-1 level vary. A five-week dynamic

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Abbreviations: bp, base pair; HRV, heart rate variability; E, endurance; E-Sp-St, endurance-speed-strength; IGFBP3, insulin-like growth factor binding protein-3; IGF1, insulin-like growth factor-1; PCR-RFLP, polymerase chain-reaction fragment length polymorphism; RER, respiratory exchange ratio; SGA, small for gestational age; SNP, single nucleotide polymorphism; Sp-St, speed-strength; UTR, untranslated region; VCO_2, carbon dioxide output; VO_2, oxygen uptake; VO_2max, maximal oxygen uptake.

endurance training led to a decrease in IGF-1 in young women (Eliakim *et al.,* 1996) and men (Jørgensen *et al.,* 1998) despite the increase in the circumference of thigh muscle. A similar decreasing impact on circulating IGF-1 is also exerted by low calorie diet without exercise (Smith *et al.,* 1987). Other studies indicated no effects of a 6-month endurance training on baseline IGF1 (Vitiello *et al.,* 1997). Also, a 12-month strength training in elderly men and women, despite a significant improvement of either maximal oxygen uptake or muscle force, did not affect IGF-1 level (Pyka *et al.,* 1994). Some authors, however, observed an increase in IGF-1 level after 2-8 weeks of dynamic training that was positively correlated with the increase in the maximal aerobic capacity (Poehlman & Copeland, 1994; Roelen *et al.,* 1997). Therefore, training intensity and fat mass may influence the level of circulating IGF-1.

Studies on polymorphisms in IGF1 gene have been few and usually conducted in the context of lesions caused by a lower IGF-1 concentration. One of polymorphisms described by Rosen *et al*., (1998) was the polymorphism of $(CA)_n$ dinucleotide repeat in a microsatellite sequence at about 1kbp before the site of the onset of transcription of *IGF1* gene. IGF-1 concentration in blood plasma differed between the genotypes. Among seven alleles (16-22 CA repeats), the most frequent one was allele 19CA at 192 bp (Rosen *et al.,* 1998). So far, studies have focused on relationships between endocrine (but not tissue) secretion with polymorphisms in the promoter. It was observed that individuals without the allele at 192 bp were significantly shorter, have a lower IGF-1 plasma level (18%), and are also more susceptible to ischemic heart disease and type II diabetes (Vaessen *et al.,* 2001). On the other hand, in a group of healthy men and women examined by Rosen *et al*., (1998) genotype 192/192 featured the lowest IGF-1 blood level. Arends in a study of SGA babies noted the presence of ten alleles with the most frequent one at 189 bp and the most frequently inherited one at 191 bp linked with the lowest IGF-1 blood level (Arends *et al.,* 2002).

The present study attempts to examine this polymorphism in the context of its potential influence on aerobic capacity with regard to the character of activity of insulin-like growth factor 1 manifested by, inter alia, mobilization of energy substrates (e.g. enhanced transportation of carbohydrates and amino acids to muscle cells), intensified gluconeogenesis in the liver, and oxidation of fatty acids. It affects both energy supply during exercise, and also perhaps indirectly, the VO_2max level.

Insulin-like growth factor-binding proteins, such as insulin-like growth factors and their receptors, play a key role in the regulation of cell proliferation and apoptosis. IGFBP-3 fulfills many functions with the most important ones being maintaining of IGF-1 and IGF-2 in the blood, modulating their bioactivity and directly inhibiting growth in extravascular tissue compartments, where the expression of *IGFBP3* takes place in a controlled fashion (Ferry *et al.,* 1999). *In vivo*, IGF-1 and IGF-2 always form a complex with one of 6 IGF binding proteins (IGFBP1-6). In the blood serum 80-85% of insulin-like growth factor-binding proteins remain in a complex of 150 kDa consisting of three components: one IGF molecule, IGFBP-3 and acid-labile subunit (ALS), which is present only in a blood serum. ALS maintains IGFBP-3/IGF in the blood vessels and prolongs the half-life of IGF in blood circulation. Its synthesis is stimulated by a growth hormone. IGFBP-3 has both an inhibitory and growth-inducing influence on cells, independent of IGF, and affects specific binding proteins or IGFBP-3 surface receptors on the cytosol side or in nuclear compartments and the extracellular matrix (Rajaram *et al.* 1997).

IGFBP3 gene consists of 5 exons with the distance of 8.9 kbp, and its product is a 264-amino acid protein chain with the mass of 28.7 kDa (Cubbage *et al.,* 1990). In 2001 a few SNP type polymorphisms were identified in the promoter region of *IGFBP3* gene. The most significant one, i.e. A>C substitution in locus -202, was strongly correlated with the level of circulating IGFBP-3 in 478 men. An in vitro experiment confirmed a greater activity of the promoter with the presence of variant A in locus -202, compared with variant C (Deal *et al.,* 2001; Cheng *et al.,* 2005). A cross-sectional study on a multiethnic population (African Americans, Hawaiians, Japanese Americans, Hispanics, Caucasians) was also conducted to determine the effects of the polymorphisms in *IGF1*, *IGFBP1* and *IGFBP3* genes on the levels of their corresponding proteins in blood. Five SNP polymorphisms (rs3110697, rs2854747, rs2854746, rs2854744, rs2132570) in *IGFBP-3* gene were strongly correlated with the protein blood level (Cheng *et al.,* 2007).

A study of the effects of the polymorphism in *IGFBP3* promoter (-202 A/C) on *IGFBP-3* blood level in response to recombinant growth hormone therapy in children with growth hormone deficiency revealed a higher IGFBP-3 level and faster increase of body height in children with genotype AA than with genotypes AC and CC (Costalonga *et al.,* 2009). Thus, this polymorphism is not only significant for medical conditions, but can also affect the genotypic variability of healthy people.

IGFBP-3 determines the bioavailability of circulating IGF-1, by influencing the metabolic function of growth factors related to the mobilization of sources of substrates for ATP resynthesis. This is why -202 A>C *IGFBP3* polymorphism with $(CA)_n$ repeats in *IGF1* promoter has been selected for the present analysis.

The aim of the present study was to analyze polymorphisms of selected candidate genes, which, as far as the circulatory system and muscle metabolism are concerned, can possibly affect physical fitness measured with VO_2max. The subjects included young people practicing sports of aerobic/anaerobic energy metabolism and non-training individuals. Research has not yet clearly confirmed whether and in what ways the mentioned polymorphisms affect physical fitness measured with VO_2max. Many studies did not examine these polymorphisms for their correlations with maximal oxygen uptake, or the results these studies remain extremely divergent. The present study is a contribution to this discussion.

MATERIALS AND METHODS

Characteristics of subjects. An analysis of association was carried out using the results of physiological and genetic studies of 239 individuals. Smokers and subjects outside the age brackets of 18–26 years and normal BMI range were excluded. Individuals for whom there were doubts as to whether their fitness test (treadmill test) results were not maximal because of their low motivation were also excluded from the study protocol. The study sample consisted of 154 male (119 training sports, 37 non-training) and 85 female (37 training sports, 48 non-training). All subjects were students of the University School of Physical Education, and thus, even the non-training individuals displayed a higher than average level of physical activity. The subjects trained endurance

Table 1. Maximal oxygen uptake (VO_2max in ml/kg·min^{-1}) mean values of female and male

Sex	NT						T					
	N	Min	Max	$\bar{X}$	SD	CV	N	Min	Max	$\bar{X}$	SD	CV
F (n=85)	48	30.60	58.40	42.29	5.16	12.19	37	33.80	59.80	49.91	5.85	11.73
M (n=154)	35	42.30	62.20	50.74	4.29	8.45	119	40.30	79.00	56.42	7.21	12.77

NT — non training group; T — training group.

sports such as the marathon, rowing, and triathlon races; sports involving energy metabolism of aerobic/anaerobic character — field hockey, volleyball, football, handball, rowing; and speed-strength sports, e.g. bodybuilding, sprints, kayaking, long jump, and high jump.

Genotyping. Genetic analyses were conducted at the Laboratory of Genetic Analyses at the University School of Physical Education in Poznań, certified by ISO 9001:2008 standards (no. 69178-2009-AQ-POL-RvA). DNA was isolated from 5 ml of peripheral blood collected from the participants onto anticoagulant (EDTA). DNA isolation was performed using guanidine isothiocyanate (GTC, Sigma) method. SNP *Alw21*I restriction site -202 A→C polymorphism in *IGF1BP3* was genotyped by PCR-RFLP analysis. DNA was amplified in a volume of 20 µl. Primers sequence was: Forward — CCA CgA ggT ACA CAC gAA Tg and Reverse — AgC CgC AgT gCT CgC ATC Tgg (Deal *et al.,* 2005). The 35 cycle reaction was run in a Biometra T-personal thermocycler. The cycle comprised initial denaturation at 95°C for 10 min, denaturation at 95°C — 30 s, annealing at 64°C — 30s, synthesis at 72°C — 30s and final synthesis at 72°C for 10 min. PCR products were separated on 2% agarose gel. Electrophoresis was run at 100 V for 30 min in Biometra agagel mini horizontal apparatus (Germany), and the results were visualized on a UV transilluminator with ethidium bromide (5mg/ml). The digested with *Alw21*I enzyme PCR products were then electrophoresed in 10% polyacrylamide gel and silver stained.

The polymorphism of $(CA)_n$ tandem repeats in *IGFI* gene promoter was analyzed using the Amersham Biosciences ALFExpress DNA analysis system due to fairly insignificant differences (2 bp) between possible consecutive polymorphic variants. The analyses were conducted in the Institute of Human Genetics of the Polish Academy of Sciences in Poznań. A mixture of 1.2 ml of PCR product with 6 ml of loading buffer with internal markers of 113 bp and 268 bp, was denaturated for 5 min. at the temperature of 94°C, and then rapidly cooled down on ice. The content of the test tube was put on gel. In the electrophoresis a denaturating 6.15% polyacrylamide gel was used with the acrylamide methylenobisacrylamide ratio of 20:1. The separation was carried out in a temperature of 55°C, at 25W with a 0.6 × TBE buffer solution until the emergence of a peak corresponding to the second internal marker (268 bp). The detection was made on the ALFExpress kit through stimulation of the luminescence of Cy5 marker indicating the PCR forward starter. The results were analyzed with the ALFExpress software.

The analysis of variance for $(CA)_n$ repeats in *IGF1* gene in women was carried with the t-test, and in men with the Kruskal-Wallis test.

VO_2max determination. Physiological analyses were conducted at the Laboratory of Functional Examinations at the University School of Physical Education in Poznań, certified by ISO 9001:2008 standards (no. 69178-2009-AQ-POL-RvA). In order to determine the maximal oxygen uptake of the participants, the direct method during exercise tests on a treadmill (Woodway, USA) was used. During each test, the composition of air inhaled and exhaled (VO_2, VCO_2, VE/MV, RER and HR) was analysed by Oxycon Mobile ergospirometer (Jaeger, Germany). The exercise tests were carried out on a treadmill with increasing load, starting from a running speed of 8 km/h, increasing the load by 2 km/h every 3 min, until the moment of maximum individual load was reached.

RESULTS

The subjects performed a treadmill test to measure their maximal oxygen uptake levels (VO_2max) directly with the use of an Oxycon Mobile ergospirometer with constant data transfer to a PC registering changes of such physiological parameters as heart rate (HR), inhaled and exhaled air volume (VO_2, VCO_2, VE/MV) and respiratory exchange ratio (RER).

The division of the sample into the training and non-training subgroups was justified by the different character of energy metabolism related to practicing individual sports. As expected, women attained lower VO_2max levels than men (Table 1). Among the training subjects, the highest maximal oxygen uptake levels were reached by athletes of endurance sports, and the lowest by athletes of speed and strength sports.

The present study involved an analysis of association of -202 A→C *IGFBP3* polymorphism and $(CA)_n$ tandem repeats of *IGF1* promoter with the maximal oxygen uptake level. The study examined the frequencies of alleles and genotypes as well as distribution of

Table 2. Descriptive statistics and comparative analysis of maximal oxygen uptake (VO_2max in ml/kg·min^{-1}) between genotypes of *Alw21I* polymorphism of *IGFBP3* gene.

IGFBP3	Sex			
	Female		Male	
	n	$\bar{X}$ ±SD (min ; max)	n	$\bar{X}$ ±SD (min ; max)
CC	32	44.68 ± 6.21 (33.8 ; 59)	58	56.23 ± 7.62 (40.3 ; 79)
CA	35	46.01 ± 59.8 (6.9 ; 31.3)	79	54.47 ± 72 (6.5 ; 42.3)
AA	15	45.88 ± 54.2 (7.1 ; 30.6)	15	52.4 ± 72.3 (6.5 ; 44.3)
p		0.6957		0.437

The statistically significant difference in maximal oxygen uptake between the given genotypes was marked by underlining at p=0.05.

Table 3. Descriptive statistics and a comparative analysis of maximal oxygen uptake (VO_2max in ml/kg·min^{-1}) values between groups of different *Alw21I* polymorphism genotypes of *IGFBP3* gene in non-training and training female and male.

IGFBP3		CC					CA					AA					p
Sex	Group	N	$\bar{X}$	S.D.	Min	Max	N	$\bar{X}$	S.D.	Min	Max	N	$\bar{X}$	S.D.	Min	Max	
F	NT	18	41.29	3.47	34.20	47.80	22	43.65	5.80	31.30	58.40	7	40.27	6.39	30.60	50.50	0.2025
	T	14	49.05	6.31	33.80	59.00	13	50.01	6.99	39.30	59.80	8	50.79	2.58	46.40	54.20	0.7992
M	NT	12	53.52 A	4.36	48.20	62.20	21	49.17 A	3.35	42.30	54.50	1	45.70	–	–	–	0.0096
	T	46	57.31	8.12	40.30	79.00	58	56.39	6.35	42.30	72.00	14	52.88	6.49	44.30	72.30	0.1296

The statistically significant difference in maximal oxygen uptake between the given genotypes was marked by underlining with the letter A at $p \leq 0.01$; NT — non training group; T — training group.

VO_2max values. The χ^2 test confirmed a normal distribution of the examined parameter, with *IGFBP3* gene in a genetic equilibrium ($\chi^2_{tab;\ n\text{-}1\ =\ 2,\ \alpha\ =\ 0.05} = 5.991$; $\chi^2_{tab;\ n\text{-}1\ =\ 2,\ \alpha\ =\ 0.01} = 9.21$). The homogeneity of variances was checked with Bartlett's test. Table 2 demonstrates descriptive statistics and a comparative analysis of VO_2max levels for polymorphic variants of studied *IGFBP3* gene. Differences between the CC and AA genotype ($p=0.05$) were observed in male group. In order to examine maximal oxygen uptake differences for different genotypes in relation to the level of physical activities, the male and female subjects were divided into a training and non-training groups. The distribution of genotypes and VO_2max values (minimal, maximal, mean) in the examined SNP are presented in Table 3. The obtained results were also examined in terms of VO_2max levels reached by athletes with different genotypes representing sports dominated by different energy metabolism patterns. The distribution of genotypes and VO_2max values (minimal, maximal, mean) in subgroups for particular polymorphisms are presented in Table 4. As for *IGFBP3 Alw21I* a significant difference between genotypes CC and AA ($p \leq 0.05$), and a highly significant difference between genotypes CA and AA ($p \leq 0.01$) were found in the subgroup of male athletes practicing endurance-speed-strength sports. The differences described earlier in the part about the subdivision of athletes into sports types of different metabolism patterns were confirmed at $p=0.05$ and $p \leq 0.01$ among the non-training men.

Analysis of the $(CA)_n$ tandem repeats of insulin-like growth factor 1

The $(CA)_n$ polymorphism of *IGF1* gene was genotyped with the use of PCR, with product separation using the ALFExpress kit. An analysis of 191 subjects (130 men, 61 women) yielded eight polymorphic variants with the lengths from 184 bp to 198 bp (the allele length is hereinafter used as the allele name). The most frequent (35%) was allele 188, and the least frequent (0.5%) was allele 196. Table 5 presents the mean values of maximal oxygen uptake reached by individuals with particular *IGF1* polymorphism genotypes. The highest VO_2max in the group of men was obtained for genotype 190/198 (the only case). The next, more numerous group with the highest mean VO_2max (62.69 ml/kg/min) were individuals with genotype 188/194. The lowest VO_2max was found in men with genotype 186/186. Among women, the highest VO_2max (56.2 ml/kg/min) was achieved for genotype 192/194, and the lowest (38.2 ml/kg/min) for an individual with genotype 188/194 (the only case). The next, more numerous group with the lowest VO_2max mean value (44.38 ml/kg/min) were women with genotype 186/186. Apart from some single cases, genotype 186/186 was the one with the lowest mean VO_2max value.

In the comparative analysis of VO_2max values of individuals with the most frequent allele 188 all observed genotypes were divided into three groups: homozygous genotypes (188/188), genotypes with only one allele 188 (188/-), and genotypes without allele 188. Bartlett's test confirmed the homogeneity of variances in the group of men and women. The analysis of variance revealed significant differences in the maximal oxygen uptake levels between genotypes 188/188 and 188/-, and 188/- and -/- in the group of women.

In the present study IGF-1 level in blood was not marked. Depending on different research studies, genotype 192/192 was either related to the highest or the lowest IGF-1 blood level. Because of the potential significance of this allele the values of maximal oxygen uptake were compared in three genotypic groups. The division of individuals with allele 192 into three groups was similar to that for the other alleles mentioned earlier: homozygous genotypes 192/192, genotypes with one allele 192 (192/-) and genotypes without allele 192 (-/-). Bartlett's test revealed the homogeneity of variances only in the group of women. T-test for women and Kruskal-Wallis test for men did not yield significant VO_2max differences between individual genotypes.

Because of the small size of the sample and too large number of genotypes, a reliable comparative analysis of VO_2max in genotypes of training and non-training subjects and in subjects practicing sports of different types of energy metabolism was not possible. Almost one half of non-training subjects had allele 186 in their genotype, while only 18% of athletes had this allele. The athletes with the highest VO_2max, who represented sports from all energy metabolism categories, often featured alleles 194 and 188. However, it is impossible to point to the allele that would most definitely determine the VO_2max.

DISCUSSION

When we began our analysis, some polymorphisms had been already widely described in literature on population of Polish athletes (Cięszczyk et al., 2011a). There had been some attempts to examine the relations between gene polymorphisms and physical fitness (Maciejewska-Karłowska 2013; Gronek & Holdys 2013, Holdys *et al.,* 2011, Leońska-Duniec 2013), or they had never been connected with VO_2max (e.g. *UCP2, UCP3,* Holdys *et al.,* 2013). Our choice of polymorphisms was based on somewhat promising research results, or great divergences of results obtained by different research

teams. To the best of our knowledge the polymorphisms investigated in the present study have never been examined on Caucasians in Poland.

IGF1 and *IGFBP3* genes had been selected for study before considering VO_2max. Although these genes obviously contribute to the development of general physical fitness through building muscle mass and mobilizing energy substrates (*IGF1)*; and regulating IGF bioavailability (*IGFBP3)*, their correlations with maximal oxygen uptake have not been examined. A few studies of polymorphisms in these genes indicate their impact on the level of circulating IGF and IGFBP-3.

IGF1 promoter polymorphism in the present study is tandem repeats of cytosine-adenine dinucleotide $(CA)_n$. The first analysis of this polymorphism by Rosen *et al.*, revealed an association of genotype 192/192 (19CA) with a lower IGF-1 blood level. Also, a 25% higher IGF-1 blood level was observed in individuals with one allele 194 than in individuals with a homozygous genotype 192/192 (Rosen *et al.,* 1998). Contrary results were attained by Vaessen *et al.*, who found the highest IGF-1 blood level in individuals with a homozygous genotype 192/192, and 18% lower in individuals without allele 192 (Vaessen *et al.,* 2001). The frequency of allele 192 in those studies may suggest it is a wild type allele. The present analysis did not involve the measurement of circulating IGF-1 level. The genotyping of promoter CA repeats of *IGF1* gene was performed on a relatively small sample of 191 individuals. The most frequent allele was allele 188 (35%), and the most frequent genotype was 188/188 (nearly 16%). A different distribution of allele frequencies compared with other studies can be related to ethnic differences and to the character of the studied group. The analysis focused on the impact of the most frequent allele — 188, and allele 192 — which is significant in terms of IGF-1 blood level — on maximal oxygen uptake levels. A significant impact ($p \leq 0.05$) of allele 188 on VO_2max in women was observed: possessing at least one allele 188 positively affected the oxygen uptake. No such impact of this allelic variant was found in men. If the activity of *IGF1* gene promoter depends significantly on allele 192 — as indicated by Rosen and Vaessen — the present study has not revealed the influence of this allele maximal oxygen uptake, although the mean VO_2max level reached by men and women with genotype 192/192 was slightly higher than that with genotype 186/186, and the frequency of allele 186 was much higher in training than non-training subjects (45%:18%). On the other hand, allele 194 was relatively frequently found in the genotype of training individuals

Table 4. Descriptive statistics and a comparative analysis of maximal oxygen uptake (VO_{2max} in ml/kg·min^{-1}) and different genotypes of *AIw21I* polymorphism in *IGBP3* gene in the groups of non-training women and men and those training various sports, subdivided according to the type of energy metabolism.

IGFBP3		CC					CA					AA					p
Sex	Group	N	$\bar{X}$	SD	Min	Max	N	$\bar{X}$	SD	Min	Max	N	$\bar{X}$	SD	Min	Max	
F	Sp-St	5	49.12	2.90	44.90	52.00	4	48.15	7.09	39.30	56.10	2	52.85	1.06	52.10	53.60	0.5220
	E-Sp-St	4	48.10	4.97	41.30	52.00	3	45.93	5.65	40.20	51.50	2	48.85	3.46	46.40	51.30	0.7875
	E	5	49.74	10.05	33.80	59.00	6	53.28	6.99	39.90	59.80	4	50.73	2.42	48.60	54.20	0.7154
	NT	18	41.29	3.47	34.20	47.80	22	43.65	5.80	31.30	58.40	7	40.27	6.39	30.60	50.50	0.2025
M	Sp-St	9	54.09	6.44	41.10	65.70	15	55.35	5.69	48.20	71.50	–					0.9662
	E-Sp-St	25	54.10 a	4.58	40.30	61.90	26	54.92 b	3.72	45.80	62.00	11	50.72 ab	3.31	44.30	56.10	0.0345
	E	12	66.40	8.46	55.30	79.00	17	59.55	8.86	42.30	72.00	3	60.80	9.97	54.50	72.30	0.1272
	NT	12	53.52 B	4.36	48.20	62.20	21	49.17 B	3.35	42.30	54.50	1	45.70	–	–	–	0.0096

The statistically significant difference in maximal oxygen uptake between the given genotypes was marked by underlining a — at $p \leq 0.05$, A, B — at $p \leq 0.01$. Sp-St: speed and strength disciplines (disciplines with predominance of anaerobic energy metabolism), E-Sp-St: endurance-speed-strength disciplines (disciplines requiring both anaerobic and aerobic energy resources), E: endurance disciplines (those predominating in aerobic energy metabolism), NT: non training group

Table 5. Maximal oxygen uptake (VO_{2max} in ml/kg·min^{-1}) mean values for most frequent *IGF1* genotypes.

IGF1	Sex				P value
	Female		Male		
	n	$\overline{X}$±S.D. (min ; max)	n	$\overline{X}$±S.D. (min ; max)	
188/188	8	49.3 ± 6.029 (41.5 ; 59.8)	22	54.57 ± 7.628 (44.9 ; 76.8)	0.0869
188/-	20	43.86 ± 5.871 (32.3 ; 54.8)	51	55.86 ± 7.315 (41.1 ; 79)	<0.001
-/-	33	47.82 ± 6.332 (34.2 ; 59)	57	54.6 ± 6.154 (40.3 ; 72.3)	<0.001

who reached the highest VO_2max. According to Rosen *et al.,* this allele increased their IGF-1 blood concentration for about 25% more than in individuals with genotype 192/192 (Rosen *et al.,* 1998). However, it was not confirmed whether allele 194 was actually related to a higher expression of IGF-1 in blood, which might have been conducive to better aerobic capacity. A component necessary for such studies would be biochemical tests that would determine IGF-1 in the plasma as well as an analysis of expression of *IGF1* gene with a different number of CA repeats. The size of the study sample did not permit a reliable statistical analysis of the influence of *IGF1* on VO_2max levels in athletes representing sports of different energy metabolism profiles.

Also, -202 A>C allele of *IGFBP3* promoter polymorphism was subject to analysis. The results indicated a higher activity of the promoter, i.e. a higher IGFBP-3 blood level for allele A than C, as manifested by more visible effects of IGFBP-3 activity in the subjects (Deal *et al.,* 2001; Costalonga *et al.,* 2009).

Significant differences in VO_2max level for -202 A>C polymorphism in *IGFBP3* gene were only found in men between genotypes *CC* and *AA* in the whole sample, and between CC and AA in athletes. The results with regard to sports of different character of energy metabolism showed that the differences were significant only for endurance-speed-strength athletes. Allele C and genotype CC were associated with higher VO_2max values, and that in view of correlation between the amount of circulating IGFBP-3 and genotype was associated with lower IGFBP-3 levels. A lower IGFBP-3 blood concentration indicates a greater concentration of IGF-1. The level of free IGF-1 is higher in athletes than in non-training individuals. This can be explained by a faster proteolysis from the complex with IGFBP-3, and by lower affinity of IGFBP3-ALS complex with IGF-1 (Eliakim *et al.,* 1996). During training the levels of IGFBP-3 and IGF-IGFBP-3-ALS complex increase (Koziris *et al.,* 1999). The present study seems to suggest that apart from exercise-induced changes in the bioavailability of IGF-1, a significant factor of differences in VO_2max could be the polymorphism in *IGFBP3* gene promoter, since these differences were found both among the male athletes and non-training subjects. Certainly a biochemical analysis would be necessary to confirm an association of maximal oxygen uptake with IGFBP-3 blood level with regard to polymorphic differences.

Some results of the present study point to a statistically significant influence of the examined polymorphisms on the VO_2max. It does not mean, however, that other polymorphisms without statistically significant results have no effect on physical fitness. They can affect other aspects of fitness or their influence might not be measured with the use of statistical tests. We did the best we could, however, to choose the most precise tests. The more or less visible tendencies in VO_2max levels for particular genotypes of examined polymorphisms correspond with the results by other research teams on different populations. Polymorphisms of *IGF1* and *IGFBP3* which are tested for their association with VO_2max seem to be an interesting contribution to this research field. Unfortunately, the study lacks a biochemical foundation which would cast more light on the results for some polymorphisms.

Studies seeking genotypes determining physical fitness have been rapidly developing on population of Polish athletes (Sawczuk *et al.*, 2013; Gronek *et al.,* 2013; Cięszczyk *et al.*, 2010; Cięszczyk *et al.,* 2011b; Sawczuk *et al.*, 2013; Cięszczyk *et al.*, 2012; Eider *et al.*, 2013; Maciejewska *et al.*, 2012; Stępień-Słodkowska *et al.*, 2013; Sawczuk *et al.*, 2012). An analysis of association of individual polymorphisms is not anymore a highly effective method of examination of the genetic background of complex traits. The currently developed genetic models involve several polymorphisms, and are aimed at the creation of optimal genetic profiles for athletes representing sports of different character of energy metabolism (Ruiz *et al.,* 2009). Analyses covering a wider range of the genome that would focus on particular aspects of physical fitness, e.g. cardio-respiratory fitness or cellular respiration efficiency, should systematically contribute to the establishment of genetic profiles most suitable for high-performance sports. The present study is a contribution to similar analyses on the Polish population and their possible application in Polish sport.

CONCLUSIONS

An association was found between -202 A/C polymorphism of *IGFBP3* gene with VO_2max in men. Higher VO_2max values were attained by men with genotype CC, especially male athletes practicing endurance sports and sports featuring energy metabolism of aerobic/anaerobic character.

A statistically significant influence of allele 188 and genotype 188/188 of tandem repeats $(CA)_n$ polymorphism of *IGF1* gene on VO_2max was found in women. Also, lower values of maximal oxygen uptake were noted in individuals with allele 186 or genotype 186/186, and higher VO_2max values in athletes with allele 194.

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The authors declare that they have no conflict of interest.

Authors contributions

All authors confirm that they have contributed to the intellectual content of this paper and have met the following requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data, (b) drafting or revising the ar-

ticle for intellectual content and (c) final approval of the published article.

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