

# NO ASSOCIATION BETWEEN tHbmass AND POLYMORPHISMS IN THE HBB GENE IN ENDURANCE ATHLETES

■ Accepted  
for publication  
10.04.2014

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**ABSTRACT:** The aim of this study was to examine the association between tHbmass and HBB gene polymorphisms in athletes of endurance disciplines. Eighty-two well-trained athletes (female n=36, male n=46), aged 19.3 ± 2.7 years, representing cross country skiing (n=37) and middle- and long-distance running (n=45), participated in the study. Genotyping for 2 polymorphisms in the HBB gene (-551C/T and intron 2, +16 C/G) was performed using restriction fragment length polymorphism analysis. Total haemoglobin mass (tHbmass) was determined by the optimized carbon monoxide rebreathing method. Blood morphology, indices of iron status (ferritin, transferrin receptor and total iron binding capacity) and C reactive protein were also determined. No differences were found in the HBB genotype and allele frequencies between male and female athletes. Regardless of the polymorphisms, no relationships were found between HBB genotypes as well as alleles and relative values of tHbmass, expressed per body mass ( $\text{g} \cdot \text{kg}^{-1} \text{ BM}$ ), both in female and male athletes. Our results demonstrated that -551 C/T and intron 2, +16 C/G polymorphisms of the HBB gene have no association with total haemoglobin mass in endurance athletes. It cannot be ruled out that several polymorphisms, each with a small but significant contribution, may be responsible for the amount of haemoglobin.

**KEY WORDS:** genetic polymorphism, HBB gene, total haemoglobin mass, athletes

## INTRODUCTION

Transport of oxygen to skeletal muscles by the cardiovascular system and its utilization in the muscle are essential factors affecting the aerobic capacity [20]. Efficient transport of oxygen to the tissues is conditioned by the size of muscle heart, blood volume and the amount of haemoglobin [20,21]. The role of haemoglobin in aerobic capacity is well documented. The results of many studies indicate a strong relationship between total haemoglobin mass (tHbmass) and maximal oxygen consumption ( $\text{VO}_2\text{max}$ ) [1,6,8,12,13]. Previous studies concluded that an increase in tHbmass by 1 g causes a rise in  $\text{VO}_2\text{max}$  of approximately  $4 \text{ ml} \cdot \text{min}^{-1}$  [21]. However, such a dependency is not observed for blood haemoglobin concentration, which is commonly used in sports diagnostics [16,24].

It has been observed that endurance athletes have higher tHbmass values than untrained subjects or power-oriented athletes [12, 15]. It is worth noting that tHbmass can also vary within endurance

disciplines, which might be caused by many factors [8, 12,21,25] including genetic predisposition [5,12,25].

So far, researchers have described 239 genes that could be related to predisposition to physical fitness and sports results [3]. One of them is the beta haemoglobin (HBB) gene [17]. However, haemoglobin genes are considered mainly due to haemoglobinopathies, which are the commonest single-gene genetic disorders in humans [10]. The impact of the HBB gene on physical performance remains unclear. The effect of this gene on aerobic capacity was described only in one study with recruits from the Chinese military police, who undertook running training for 18 weeks [11]. The authors suggested that homozygosity for the C allele of -551C/T and intron 2, +16 C/G polymorphisms may determine a higher concentration of haemoglobin in the blood and explain part of the individual variation in the cardiovascular adaptation to endurance training [11]. However, there is still no information concerning the

relationships between the HBB gene and the amount of haemoglobin in the blood; therefore the aim of this study was to examine the association between tHbmass and HBB gene polymorphisms in athletes of endurance disciplines.

## MATERIALS AND METHODS

**Subjects.** Eighty-four athletes, both male and female, from cross-country skiing and middle- and long-distance running, volunteered for the study. The subjects were given a medical and biochemical examination in order to exclude individuals who showed symptoms of infectious, cardiovascular diseases, latent iron deficiency ( $n=2$ ) or iron deficiency anaemia. Finally, the results from 82 athletes, i.e. 36 females and 46 males, were analysed. Most of the study participants were members of national junior or senior teams. All athletes were Caucasians. The study was approved by the Institute of Sport's Committee of Ethics and written informed consent was obtained from individuals.

### Study design

The study consisted of the two stages performed on one day in the following order: 1) venous blood sampling, 2) evaluation of tHbmass.

### Blood collection and analysis

The blood samples were withdrawn from the antecubital vein in the morning in a preprandial state after staying at least 15 min in a sitting position.

### Indices of iron status

Haemoglobin concentration (Hb), haematocrit (Hct), erythrocyte count (RBC), and percentage of reticulocytes (Ret) were determined by using an ADVIA 120 haematological analyser (Siemens, Germany). In blood serum the following assays were conducted: soluble transferrin receptor (sTfR) concentration by using immunoenzymatic commercial kits (Ramco, USA); ferritin concentration by using an immunoenzymatic method (Pentra, USA); total iron binding capacity (TIBC) by using a colorimetric method (BioMaxima, Poland); and C-reactive protein (CRP) by using an immunoturbidimetric method (Pentra, USA).

### DNA isolation and HBB polymorphism typing

Genomic DNA was extracted from whole blood using the GeneMATRIX Quick Blood DNA Purification Kit (Eurx, Germany) following the manufacturer's instructions. HBB gene intron 2, +16C/G and -551C/T polymorphisms were analysed by PCR-RFLP methods using primer sequences and method conditions described previously [11] with our modification. Briefly, polymerase chain reactions were carried out in 20  $\mu$ l of solution containing a standard PCR buffer, 200 ng of tested DNA, 1 U Taq Polymerase (Invitrogen, Brazil), 2 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTPs (Invitrogen, Brazil), and 1  $\mu$ M of each primer. Amplifications were performed under the following conditions: 94°C, 3 min; 35 cycles of 30 s at 94°C; 30 s

at 58°C (for intron 2, +16C/G typing) or 57°C (for -551C/T typing); 60 s at 72°C. The last elongation step was extended to 7 min. PCR products were digested with 2 U of *Ava*II restriction enzyme (Fermentas, USA) for 2.5 h at 37°C (for intron 2, +16C/G typing; G allele was cut) or with 2 U of *Rsa*I restriction enzyme (Fermentas, USA) for 2.5 h at 37°C (for -551C/T typing; T allele was cut). All digested products were electrophoresed on 3% agarose gel and visualized in a UV transilluminator using ethidium bromide staining.

### Determination of tHbmass

tHbmass was assessed using a modified version of the CO rebreathing procedure, according to Schmidt and Prommer [19,22]. Briefly, this procedure comprised inhalation of a bolus of 99.9% chemically pure CO (Linde Gas) in a dose of 1.0 ml·kg<sup>-1</sup> body mass for males and 0.8 ml·kg<sup>-1</sup> body mass for females. The samples of the arterialized capillary blood (Finalgon®, Boehringer Ingelheim, Germany) were taken from the earlobe three times: directly before the test and in the 6th and 8th minute after the respiration through the spirometer was started. Measurements of the percentage value of carboxyhaemoglobin (HbCO%) (ABL 80 Flex, Radiometer, Denmark) were performed in triplicate samples before and in the 8th minute, and in duplicate samples in the 6th minute, of the study. The detailed description of this method was given in publications by its authors [19,22]. In all participants measurements of tHbmass were done in duplicate. The typical error (TE) in our laboratory with 27 duplicated measures (24-48 h time lag between the tests) was 1.59%.

### Statistical analysis

All the data are presented as means and standard deviations, and were analysed using the Statistica 10 software package (StatSoft Inc. Tulsa, USA). Differences between mean values of tHbmass in groups of athletes possessing different genotypes of the HBB gene were tested (separately in males and females) by the Kruskal–Wallis test, whereas the Mann–Whitney U test was used in comparison of mean values of tHbmass in groups distinguished according to alleles. The significance of differences in genotype and allele frequencies as well as conformity with the Hardy–Weinberg principle was calculated using the  $\chi^2$  test. The statistical significance was set at  $p<0.05$ .

## RESULTS

The physical characteristics of subjects, separated by gender, as well as basic data concerning sports experience, training load and indices of iron status, are shown in Table 1.

Both polymorphisms were in Hardy–Weinberg equilibrium in male and female athletes. No differences were found in the HBB genotype and allele frequencies between male and female athletes (Table 2). The HBB genotypes had no significant effect on relative values of tHbmass (Table 3). No relationships were found between HBB alleles in the case of both polymorphisms and relative values of tHbmass (Figures 1 and 2).

**TABLE 1.** CHARACTERISTICS OF STUDY PARTICIPANTS (MEAN ± SD)

Variable	Females (n=36)	Males (n=46)
Age (years)	20.1 ± 3.4	18.7 ± 1.7
Body height (m)	1.7 ± 0.1	1.8 ± 0.05
Body mass (kg)	57.0 ± 5.4	68.4 ± 6.8
Training experience (years)	6.3 ± 2.7	5.7 ± 3.1
Training/week (h)	13.5 ± 6.9	14.2 ± 5.4
Haemoglobin (g · dl <sup>-1</sup> )	13.8 ± 0.8	15.7 ± 0.7
Haematocrit (l · l <sup>-1</sup> )	40.7 ± 2.7	45.1 ± 2.4
RBC (x 10 <sup>12</sup> · l <sup>-1</sup> )	4.7 ± 0.3	5.3 ± 0.3
Ferritin (ng · ml <sup>-1</sup> )	34.1 ± 24.6	51.1 ± 33.7
sTfR (mg · l <sup>-1</sup> )	5.0 ± 1.1	4.8 ± 0.9
TIBC (mg · dl <sup>-1</sup> )	324 ± 26.3	313.8 ± 34.4

**TABLE 2.** GENOTYPE AND ALLELE FREQUENCIES OF INTRON 2, +16 G/C AND -551C/T POLYMORPHISMS OF HBB GENE IN MALE AND FEMALE ATHLETES

		intron 2, +16 C/G				
		genotypes			alleles	
		CC	CG	GG	C	G
Female (n=36)	n	3	16	17	22	50
	%	8.3	44.4	47.2	30.6	69.4
Male (n=46)	n	1	15	30	17	75
	%	2.2	32.6	65.2	18.5	81.5

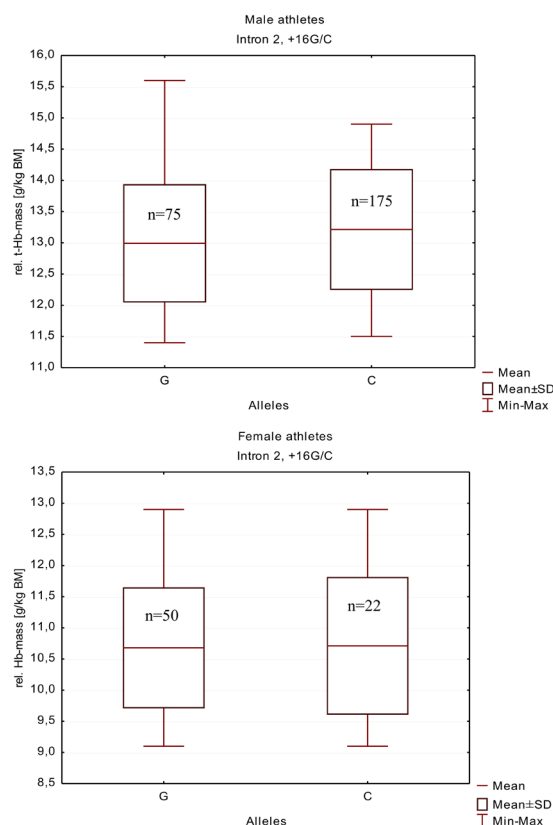
  

		-551 C/T				
		genotypes			alleles	
		CC	CT	TT	C	T
Female (n=36)	n	8	20	8	36	36
	%	22.2	55.6	22.2	50.0	50.0
Male (n=46)	n	4	24	18	32	60
	%	8.7	52.3	39.1	34.8	65.2

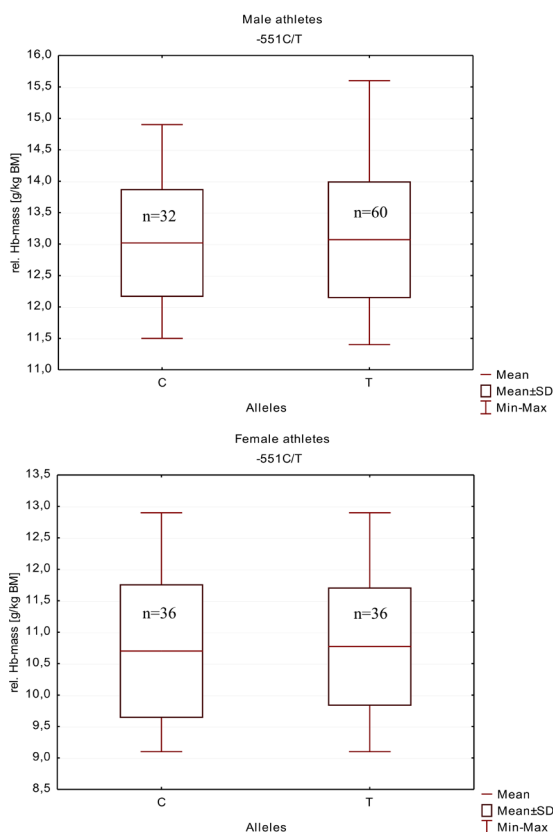
**DISCUSSION**

This study was the first to determine the association of tHbmass and the HBB gene in athletes. The main finding of the present study was no relationships between tHbmass and -551 C/T, or intron 2, +16 C/G polymorphisms in the HBB gene, either genotypes or alleles.

No differences were found in the HBB genotype distribution between male and female athletes. Due to the limited extent of research in this area, it is difficult to compare our results to others. In the present study the frequency of HBB variants in males was different from that observed in the untrained Chinese male population [11], which may result from ethnic origin or selection for endurance disciplines [11,27]. A lack of differences in genotype



**FIG. 1.** RELATIVE THBMASS ACCORDING TO ALLELES OF INTRON 2, +16 G/C POLYMORPHISM OF HBB GENE IN MALE (UPPER) AND FEMALE (BOTTOM) ATHLETES



**FIG. 2.** RELATIVE THBMASS ACCORDING TO ALLELES OF -551C/T POLYMORPHISM OF HBB GENE IN MALE (UPPER) AND FEMALE (BOTTOM) ATHLETES

**TABLE 3.** TOTAL HAEMOGLOBIN MASS ACCORDING TO HBB GENOTYPES IN MALE AND FEMALE ATHLETES (MEAN  $\pm$  SD)

		intron 2, +16 C/G			Kruskal-Wallis test
Female (n=36)	CC (n=3)	CG (n=16)	GG (n=17)	p=0.57	
	11.1 $\pm$ 0.8	10.6 $\pm$ 1.2	10.7 $\pm$ 0.8		
Male (n=46)	CC (n=1)	CG (n=15)	GG (n=30)	not tested	
	13.8	13.2 $\pm$ 1.0	12.9 $\pm$ 0.9		
		-551 C/T			
Female (n=36)	CC (n=8)	CT (n=20)	TT (n=8)	p=0.72	
	10.5 $\pm$ 1.1	10.8 $\pm$ 1.1	10.8 $\pm$ 0.5		
Male (n=46)	CC (n=4)	CT (n=24)	TT (n=18)	p=0.29	
	12.4 $\pm$ 1.0	13.1 $\pm$ 0.8	13.0 $\pm$ 1.1		

distribution of -551 C/T polymorphism between alpine skiing athletes and untrained persons (control group) was described only in conference abstracts [18].

The HBB gene determines the structure of  $\beta$  chains of polypeptide in adult haemoglobin Hb A. Polymorphism -551 C/T of this gene is in the region which functions as a silencer in transient expression [7,11]. In turn, intron 2 +16 C/G of this gene may lead to different expression, because it is in the alternative splicing region of mRNA [11,23]. Simultaneously, it is suggested that the homozygous CC for both polymorphisms could have a higher haemoglobin concentration [11]. Because haemoglobin is the basic transporter of oxygen, a high total amount of it, in large part, determines high maximal oxygen uptake ( $VO_{2max}$ ) [1,13]. The results of He et al. [11] indicated a lack of association of these polymorphisms and  $VO_{2max}$ ; however, homozygotes for intron 2, +16 CC and -551 CC had a decreased oxygen cost of running compared to the other genotypes, which suggested that C alleles were conducive to aerobic capacity [11]. Although in the present study aerobic indices were not examined, our results did not confirm an association between C alleles in both polymorphisms and haemoglobin amount. However, it cannot be excluded that the possible reason for a lack of relationships between both polymorphisms of the HBB gene and tHbmass was due to small sample size, especially in groups with CC genotypes. It is worth noting that haemoglobin amount is a complex trait likely not reducible to double polymorphisms. Furthermore, epigenetic factors, the environment

and the complex gene-gene and gene-environment interactions are also important determinants [2,14,26]. For example, Pro582Ser polymorphism of the HIF1 $\alpha$  gene affects red blood cells, iron homeostasis [26] and physical performance [4].

## CONCLUSIONS

The present study showed that -551 C/T and intron 2, +16 C/G polymorphisms of the HBB gene were not determinants of total haemoglobin mass, either in female or in male Polish endurance athletes. It is more likely that several polymorphisms, each with a small but significant contribution, are responsible for haemoglobin amount. Further research including tHbmass, genes and aerobic indices on a large population of athletes is necessary to better understand the relationship between haemoglobin amount, genetic predisposition and physical performance.

## Acknowledgements

Funding: The study was financially supported by the Ministry of Science and Higher Education of the Republic of Poland (grant no. N N404 52 52 38) and by the Ministry of Sport and Tourism of the Republic of Poland from the Fund for Development of Physical Culture (agreement number 2013/0209/0223/SubB/DSW/DK/JD).

**Conflict of interest:** The authors declare that they have no conflicts of interest concerning this article.

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