Molecular Research on the Effect of IL10 Gene Polymorphism on Obesity Parameters in Highly Physically Active Young Men

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Abstract

The main aim of this study was to investigate the association between 5 polymorphisms of the interleukin 10 (IL10) gene with body composition parameters in physically active young men. A cohort of 131 male students was enrolled and the following IL10 SNPs were analyzed: rs1518111, rs1878672, rs3024496, rs3024498 and rs3024505. The subjects were divided into groups depending on obesity parameters: body mass index (BMI) and fat percentage (fat %). Statistical analysis was conducted for a single locus and haplotypes, an association between SNPs and body composition parameters were tested with four genetic models: dominant, recessive, codominant and overdominant mode of inheritance (MOI). The results showed statistically significant association with BMI for CCGTA haplotype in additive model (score = -2.00, p = 0.04) and in dominant model (score = -2.30, p = 0.02). The obtained results indicate the significant participation of IL10 polymorphisms in the regulation of body weight in physically active people.

1. Introduction

World Health Organization (WHO) currently describes overweight and obesity as abnormal or excessive fat accumulation that may impair health, as identified by body mass index (BMI), which relates to person's weight in kilograms divided by the square of height in meters (kg/m²). An overweight person is one whose BMI is equal to or greater than 25 whereas obese is a person for whom his BMI is equal or exceed 30, despite the age or gender. Prevalence of obesity between 1975 and 2016 nearly tripled. In 2016 according to WHO data 39% of adults aged 18 years and over and 13% of the world's adult population were overweight or obese, respectively. The main cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. It results from an increased intake of energy-dense foods being rich in sugars and fat and a decrease in physical activity as a consequence of sedentary work, changes in modes of transportation or increased urbanization [1]. Obesity is associated with chronic low-grade inflammation state as an physiological response necessary to restore homeostasis disrupted by environmental factors. Apart from these environmental factors, also genetic factors are of profound importance. Obesity is considered a multifactorial trait, as over 700 genes and chromosomal regions have been specified as having influence in body weight and metabolic regulation where obesity is only one of the symptoms of congenital genetic disorders or polygenic, associated with the presence of polymorphisms in several genes [2].

The relationship between obesity and interleukin-mediated inflammatory process has been under investigation for many years. Balistreri et al. (2010) believe that the relationship between obesity and obesity-related inflammatory diseases can be explained by evolutionary speculation, e.g. the ability to resist hunger and elicit an effective immune response to pathogens [3]. Depending on the severity of metabolic disorders, people with obesity can be divided into two categories. The first group includes people with mild disorders and low expression of pro-inflammatory factors. On the other hand, in the second group, there is a significant increase in pro-inflammatory factors and we can observe in this group lot of metabolic dysfunction and disorders in processes controlling metabolism. Trayhurn and Wood (2004) proposed possible explanations of this situation. The first possibility is the release of pro-inflammatory agents from organs other than white adipose tissue, e.g. the liver. The second theory assumes that white adipose tissue secretes factors stimulating production of inflammatory markers in the liver and other organs. The third possible explanation is that the adipocytes themselves increase production of some or most inflammatory markers [4]. Cellular composition of adipose tissue defines its secretory function. The adipose tissue consists of adipocytes, lymphocytes, macrophages, mast cells, eosinophils, fibroblasts and cells of the blood vessel wall. The number of individual types of cells, their phenotype and distribution determine the type of adipose tissue and the degree of obesity. Macrophages in lean mice and humans make up around 5% of the cells in adipose tissue, during obesity they constitute up to 50% of all adipose tissue cells. Type II macrophages are present in the adipose tissue of lean people, while type I macrophages appear in people with obesity. Macrophages of type II are responsible for tissue remodeling and inflammation resolution [5]. 'Classically activated' M1 macrophages, leads to increased expression levels of TNFs and inducible nitric oxide synthase (iNOS) and the release of nitric oxide (NO). The pro-inflammatory cytokines IL12 and IL23 are likewise produced, while synthesis of the anti-inflammatory cytokine IL10 is reduced [6]. Macrophages of type II are involved in the repair of damaged tissues and prevent the development of inflammation. These macrophages have been shown to secrete significant amounts of IL10 with a simultaneous decrease in the synthesis of IL12 and IL23 [7].

The IL10 gene is located on chromosome 1 at 1q31-1q32, comprised of 4 introns and 5 exons flanked by untranslated regions (UTR), spanning approximately 5.2 kb [8]. The IL10 gene is surrounded upstream by other members of the IL10 family of cytokines IL19, IL20 and IL24, and downstream by the Mapkapk2 gene. IL10 is express in many types of cells within both the innate (including macrophages, monocytes, dendritic cells, mast cells, neutrophils, eosinophils and natural killer cells) and adaptive (including CD4+ T cells, CD8+ T cells and B cells) immune systems [2, 9]. Many studies have shown that polymorphisms in the coding and regulatory non-coding regions of the IL10 gene have a significant impact on the expression level and functionality of the cytokine IL10, which is associated with susceptibility to the development of a number of diseases based on the inflammatory state. Xia et al. (2018) showed a relationship between the rs1518111 polymorphism and spastic tetraplegia in patients with cerebral palsy [10]. By contrast, Shahriyari et al. (2019) showed that the same polymorphism likely plays a protective role in Behcet's disease [11]. Tsilidis et al. (2009) showed the relationship between the rs3024496 and rs3024498 polymorphisms and colorectal cancer [12]. Lin et al. (2017) found a significant association between the rs3024496 genotypes and IBD in children; and the association between the prevalence of the rs3024498 SNP genotype with IBD in adults and children [13]. IL10 may also be involved in controlling of body mass. Esposito et al. (2003), tested premenopausal women with obesity (n = 50) and age-matched women (n = 50) with normal weight [8]. He observed elevated circulating IL10 level in the obese group, however, low IL10 levels were observed in group with metabolic syndrome. In another study, Liu et al. (2018) observed lower IL10 levels in serum of children with obesity and hypertriglyceridemia [9]. Immune mechanisms may play a potential role in the development and maintenance of obesity and only few studies have investigated the relationship between excess body weight and polymorphisms in the IL10 gene. However, the exact relation between IL10 gene polymorphisms and the risk of obesity has not been clearly defined.

As both environmental and genetic factors are involved in the pathogenesis of obesity, environmental factors may mask genetic factors, which may be critical to the results of genetic analysis. Therefore, the aim of this study was to investigate the frequencies of polymorphisms of IL10 gene and the relationship between IL10 gene polymorphisms and the body composition parameters (fat % and BMI) in homogenous population of physically active overweight and non-overweight young men residing under unified environmental conditions.
2. Results

Among 131 investigated individuals, 39 had BMI value exceeding 25 (OVER\textsubscript{BMI}) and for 20 of them fat content exceeded 20% (OVER\textsubscript{Fat}). Mean BMI in OVER\textsubscript{BMI} was 27.2 ± 1.6 and mean fat share in OVER\textsubscript{Fat} was 22.9 ± 2.4%. Significant differences (p < 0.05) were found between the control group CON\textsubscript{BMI} and the study group OVER\textsubscript{BMI} regarding such features as weight, BMI, fat %, BMR (Basal Metabolic Rate) and total water content in the body and values of most of these variables established for OVER\textsubscript{BMI} exceeded the values for CON\textsubscript{BMI}. No significant differences appeared in age and height (p = 0.42 and 0.45, respectively).

Similarly, significant differences were established for weight, BMI, fat%, BMR and total water content between OVER\textsubscript{Fat} and CON\textsubscript{Fat}. Only values of BMR and total water content were lower in OVER\textsubscript{Fat} than in CON\textsubscript{Fat} whereas for other variables their values in OVER\textsubscript{Fat} exceeded values for CON\textsubscript{Fat} (Table 1).

| Group         | All (n = 131) | OVER\textsubscript{BMI} (n = 39) | CON\textsubscript{BMI} (n = 92) | OVER\textsubscript{Fat} (n = 20) | CON\textsubscript{Fat} (n = 111) |
|---------------|---------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Age           | 22.4 ± 2.2    | 22.6 ± 2.5                      | 22.3 ± 2.1                      | 22.3 ± 2.3                      | 22.4 ± 2.2                      |
| Height [cm]   | 179.9 ± 6.6   | 180.5 ± 7.3                     | 179.6 ± 6.3                     | 181.0 ± 8.3                     | 179.6 ± 6.2                     |
| Weight [kg]   | 78.9 ± 9.8    | 88.7 ± 8.5*                     | 74.7 ± 6.9*                     | 91.2 ± 9.6*                     | 76.7 ± 8.0*                     |
| BMI [pts]     | 24.3 ± 2.4    | 27.2 ± 1.6*                     | 23.1 ± 1.4*                     | 27.8 ± 1.9*                     | 23.7 ± 1.8*                     |
| Fat %         | 16.3 ± 4.2    | 20.9 ± 2.9*                     | 14.3 ± 2.8*                     | 22.9 ± 2.4*                     | 15.1 ± 3.2*                     |
| BMR           | 2763.1        | 3791.9*                         | 2326.9*                         | 2492.9*                         | 2811.7*                         |
| Total water [%]| 60.6 ± 3.4   | 57.1 ± 2.7*                     | 62.1 ± 2.5*                     | 55.5 ± 2.2*                     | 61.5 ± 2.6*                     |

* statistically significant (p < 0.05) difference in Student T test between CON and OVER groups. BMI - body mass index. BMR - basal metabolic rate.

Selected \textit{IL10} gene SNPs and their variations are presented Fig. 1. and in Table 2. Genotype frequencies in OVER\textsubscript{BMI} group were in Hardy–Weinberg equilibrium, although the observed rs3024498 (\textit{IL10}) genotype frequencies differed significantly from expectations (p = 0.03). The genotypes frequency at the threshold of statistical significance were observed for rs1878672 and rs3024496 for the Hardy-Weinberg equilibrium in the OVER\textsubscript{Fat} group (p = 0.05 and p = 0.05 respectively) (Table 3).

Table 2

| SNP ID (RefSNPs) | Chromosomal position | Variation | Gene location | Functional consequence |
|------------------|----------------------|-----------|---------------|------------------------|
| rs1518111        | 1:206771300 (GRCh38) | c.T > C   | Intron        | upstream transcript variant |
| rs1878672        | 1:206770368 (GRCh38) | c.G > C   | Intron        | upstream transcript variant |
| rs3024496        | 1:206768519 (GRCh38) | c.A > G   | non coding transcript | 3 prime UTR variant |
| rs3024498        | 1:206768184 (GRCh38) | c.T > C   | non coding transcript | 3 prime UTR variant |
| rs3024505        | 1:206766559 (GRCh38) | c.G > A   | downstream regulatory sequence | expression influence |

Table 3

| SNP      | MAF(%) | OVER\textsubscript{BMI}+CON\textsubscript{BMI} | OVER\textsubscript{BMI} | CON\textsubscript{BMI} | OVER\textsubscript{Fat} | CON\textsubscript{Fat} |
|----------|--------|-----------------------------------------------|--------------------------|------------------------|--------------------------|------------------------|
| IL10 (rs1518111) | Allele T 26.72 | 1 | 0.66 | 0.79 | 0.59 | 0.81 |
| IL10 (rs1878672)  | Allele C 45.42 | 0.16 | 0.17 | 0.54 | 0.05 | 0.57 |
| IL10 (rs3024496)  | Allele G 44.66 | 0.11 | 0.17 | 0.30 | 0.05 | 0.34 |
| IL10 (rs3024498)  | Allele C 22.48 | 0.08 | 0.03* | 0.59 | 0.54 | 0.15 |
| IL10 (rs3024505)  | Allele A 16.79 | 0.53 | 0.41 | 0.18 | 0.15 | 0.19 |

* statistically significant (p < 0.05). MAF – minor allele frequency.

Genotypes \textit{IL10} rs1518111, \textit{IL10} rs1878672, \textit{IL10} rs3024496, \textit{IL10} rs3024498 were not associated with BMI (Table 4). Analysis of the link between \textit{IL10} rs3024505 and BMI showed significant association of this SNP with BMI values exceeding 25 (Table 5). Associations were found for the overdominant genetic model (G/G + A/A vs. A/G) with OR values = 0.39, 95% CI 0.16–0.98, p = 0.04 (Table 4). In this model the genotype A/G of rs3024505 was 2.5 times lower than G/G + A/A genotypes (p = 0.06) (Table 5).
Table 4
Association analysis of the *IL10* gene rs1518111 polymorphism with BMI.

| Genotypes and alleles | OVERBMI | CONBMI |
|-----------------------|---------|--------|
| C/C (%)               | 23 (59.0) | 47 (51.1) |
| C/T (%)               | 13 (33.3) | 39 (42.4) |
| T/T (%)               | 3 (7.7) | 6 (6.5) |
| C (%)                 | 59 (75.6) | 133 (72.3) |
| T (%)                 | 19 (24.4) | 51 (27.7) |

OR (95% CI) p-value

| Genetic models | OVERBMI vs. CONBMI |
|----------------|--------------------|
| Dominant       | 0.73 (0.34–1.55) p = 0.41 |
| Recessive      | 1.38 (0.65–2.94) p = 0.41 |
| Overdominant   | 0.68 (0.31–1.49) p = 0.33 |
| Codominant     | 1.02 (0.23–4.46) p = 0.62 |
| Allelic        | 1.19 (0.63–2.33) p = 0.68 |

OR – odds ratio. 95% CI – confidence intervals

Table 5
Association analysis of the *IL10* gene rs3024505 polymorphism with BMI.

| Genotypes and alleles | OVERBMI | CONBMI |
|-----------------------|---------|--------|
| G/G (%)               | 31 (79.5) | 58 (63.0) |
| A/G (%)               | 7 (17.9) | 33 (35.9) |
| A/A (%)               | 1 (2.6) | 1 (1.1) |
| G (%)                 | 69 (88.5) | 149 (81.0) |
| A (%)                 | 9 (11.5) | 35 (19.0) |

OR (95% CI) p-value

| Genetic models | OVERBMI vs. CONBMI |
|----------------|--------------------|
| Dominant       | 0.44 (0.18–1.07) 0.06 |
| Recessive      | 2.27 (0.94–5.50) 0.06 |
| Overdominant   | 0.39 (0.16–0.98) 0.04 |
| Codominant     | 0.40 (0.16–1.00) 0.10 |
| Allelic        | 1.8 (0.7–4.49) 0.19 |

OR – odds ratio. 95% CI – confidence intervals
The same analysis was made for association between *IL10* gene polymorphisms and fat %. The only significant association was found for *IL10* (rs3024505) in the codominant model (G/G vs. A/G) with OR = 0.22, 95% CI 0.05–1.02, p = 0.04 and in overdominant model (G/G + A/A vs. A/G) with OR = 0.21, 95% CI 0.05–0.97, p = 0.02 (Table 6).

In the codominant model the genotype A/G of rs3024505 was 4.5 times lower than G/G genotype (p = 0.06) in the OVER_Fat group. In the overdominant model the genotype A/G was over 4.7 times lower than G/G + A/A (p = 0.03) (Table 6).

### Table 6

Association analysis of the *IL10* gene rs3024505 polymorphism with fat %.

| Genotypes and alleles | OVER_Fat | CON_Fat |
|-----------------------|----------|---------|
| G/G (%)               | 17 (85.0)| 72 (64.9)|
| A/G (%)               | 2 (10.0)| 38 (34.2)|
| A/A (%)               | 1 (5.00)| 1 (0.90)|
| G (%)                 | 36 (90.0)| 182 (82.0)|
| A (%)                 | 4 (10.0)| 40 (18.0)|

| OR (95% CI) p-value | OVER_Fat vs. CON_Fat |
|---------------------|----------------------|
| Dominant            |                      |
| G/G vs. A/G + A/A   | 0.33 (0.09–1.18) 0.06|
| A/G + A/A vs. G/G   | 3.07 (0.85–11.13) 0.06|
| Recessive           |                      |
| G/G + A/G vs. A/A   | 5.79 (0.35–96.57) 0.25|
| A/A vs. G/G + A/G   | 0.17 (0.01–2.88) 0.25|
| Overdominant        |                      |
| G/G + A/A vs. A/A   | 0.21 (0.05–0.97) 0.02|
| A/G vs. G/G + A/A   | 4.68 (1.03–21.26) 0.02|
| Codominant          |                      |
| G/G vs. A/G         | 0.22 (0.05–1.02) 0.04|
| G/G vs. A/A         | 4.24 (0.25–71.18) 0.04|
| Allelic             |                      |
| G vs. A             | 1.97 (0.65–8.06) 0.31|
| A vs. G             | 0.51 (0.12–1.53) 0.31|

OR – odds ratio. 95% CI – confidence intervals

Polymorphisms *IL10* rs1518111, *IL10* rs1878672, *IL10* rs3024496, *IL10* rs3024498 and *IL10* rs3024505 haplotypes of these polymorphisms were analysed. Only haplotypes with frequency over 5% were considered. Most common haplotype was CGATG (0.26%, *IL10* (rs1518111) C > T, *IL10* (rs1878672) G > C, *IL10* (rs3024496) A > G, *IL10* (rs3024498) T > C and *IL10* (rs3024505) G > A). Statistically significant association with BMI was found for CCGTA haplotype in additive (score = -2.00, p = 0.04) and dominant model (score = -2.30, p = 0.02). No significant association was found for haplotypes and fat %. For CCGTA haplotype the chance of being CON_BMI was over 2 times greater than being OVER_BMI (Table 7).
One of analyzed polymorphisms, rs1518111 C/T, have been previously linked to a lowering expression of IL-10 mRNA, have allelic association with several BMI values. Our research showed the possible relationship of the haplotype CCGTA with maintaining normal body weight, as its incidence in the CON group was more than two times frequent than in the OVER group for the codominant model, and that the odds of being overweight for A/G were more than 4.5 times lower than for G/G (p = 0.01, OR = 0.09). These data support previous reports that polymorphisms of genes encoding interleukins may contribute to an increased risk of obesity.

Excess body weight may increase the chance for the development of chronic inflammation accompanied by the secretion of many pro-inflammatory factors. It has been observed that adipose tissue in obese individuals secretes mainly pro-inflammatory cytokines, i.e., TNF, IL6, leptin, visfatin, resistin, angiotensin II and plasminogen activator inhibitor 1, whereas adipose tissue of lean individuals secretes anti-inflammatory adipokines such as adiponectin, as well as transforming growth factor (TGF), interleukin (IL) 10, IL4, IL13, IL1 receptor antagonist (IL1RA) and apelin. Polymorphisms in the genes of pro- and anti-inflammatory cytokines and/or their receptors may exacerbate cytokine imbalances and thus contribute to the development or worsening of obesity.

Single nucleotide polymorphisms (SNPs) in cytokine genes or regions in their close proximity are considered to be important in genetic control of the production of cytokines, including IL-10. IL-10 as anti-inflammatory cytokine plays an important role in the regulation of the immune system by decreasing cytokine production, inhibiting matrix-degrading metalloproteinases, and promoting the switching of lymphocytes to the Th2 phenotype. Furthermore, IL-10 exerts essential control over the biochemical parameters such as LDL, VLDL, HDL, triglycerides and glucose level. An increase in IL-10 expression was observed in adipose tissue of obese humans and rodents, which is consistent with the findings that obesity is associated with increased levels of circulating IL-10. Hence, it is important to identify and characterize the regulation of IL-10 in obesity, especially as it has been shown that polymorphism of genes encoding interleukins may contribute to an increased risk of obesity.

The aim of this study was to investigate the frequencies of polymorphisms of IL10 gene (rs1518111, rs1878672, rs3024496, rs3024498 and rs3024505) and the relationship between IL10 gene polymorphisms and the body composition parameters (fat % and BMI) in physically active young men. Our research showed the possible relationship of the haplotype CCGTA with maintaining normal body weight, as its incidence in the CON group was more than two times frequent than in the OVER group (additive model p = 0.04 and dominant model p = 0.02). These data support previous reports that polymorphisms and allele variants of cytokine genes may be associated with obesity and may play an important role in body weight regulation. Our association analysis of the IL10 gene rs3024505 polymorphism with body fat percentage was statistically significant in the codominant model (p = 0.04) and the overdominant model (p = 0.02). In-depth tests showed that the odds of being OVERfat for A/G were more than 4.5 times lower than for G/G (p = 0.06) for the codominant model, and that the odds of being OVERfat for A/G were over 4.7 times lower than for G/G-A/A (p = 0.03) in the overdominant model.

One of analyzed polymorphisms, rs1518111, have been previously linked to a lowering expression of IL-10 mRNA, have allelic association with several other variants of IL10 polymorphisms and is an indicator of poor outcome and enhanced systemic inflammation in patients with acute coronary syndrome.

### Table 7

Haplotypes distribution and its association of IL10 with BMI.

| Haplotypes | Haplotype Frequencies % | additive |
|------------|-------------------------|----------|
|            | score = 17.00, p-value = 0.01 |
| IL10 rs1518111 | IL10 rs1878672 | IL10 rs3024496 | IL10 rs3024498 | IL10 rs3024505 | OVERBMI+CONBMI | OVERBMI | CONBMI | OR | 95% CI | score | p-value |
| C          | C          | G          | T          | A          | 0.16       | 0.09       | 0.18       | 0.44       | 0.16       | 1.21       | -2.00   | 0.24 |
| T          | G          | A          | T          | G          | 0.24       | 0.22       | 0.26       | 0.72       | 0.32       | 1.60       | -0.66   | 0.02 |
| C          | C          | G          | C          | G          | 0.23       | 0.21       | 0.23       | 0.74       | 0.35       | 1.58       | -0.33   | 0.02 |
| C          | C          | G          | T          | G          | 0.06       | 0.07       | 0.06       | 1.10       | 0.36       | 3.35       | 0.38    | 0.02 |
| T          | G          | A          | C          | G          | 0.02       | 0.02       | 0.02       | 1.49       | 0.17       | 13.41      | 0.65    | 0.02 |
| C          | G          | A          | T          | G          | 0.26       | 0.27       | 0.24       | 1.00       | NA         | NA        | 0.65    | 0.02 |

OR – odds ratio. 95% CI – confidence intervals. NA – not applicable

### 3. Discussion

In the pathogenesis of obesity both genetic and environmental factors play a significant role. Due to the extensive influence of various environmental factors, determining the influence of genetic factors on obesity or overweight susceptibility can be complicated. To limit the influence of varying environmental factors, this study selected a fairly homogeneous and environmentally isolated group of individuals. The study group consisted of unrelated male military cadets based in dormitories of a military university in Warsaw. All of them have undergone fitness tests both during recruitment to the military college and each year during their service, to ensure an appropriate level of physical fitness. Moreover, sports activities were essential part of their education and daily routine. They usually ate their meals in the university canteen, so their habitual diet was not diversified. Provided meals were balanced in terms of nutritional value and energetic value and they complied the nutritional recommendation for military professionals. In study group only in 29.8% of participants values of BMI exceeding 25 were observed. Excessive storage of fat in the body exceeding 20% was observed only in 15.3% of participants. Although the size of the study group was rather small, its homogeneity allowed us to reduce the influence of environmental factors which increased the significance of the presented analysis.

Excess body weight may increase the chance for the development of chronic inflammation accompanied by the secretion of many pro-inflammatory factors. It has been observed that adipose tissue in obese individuals secretes mainly pro-inflammatory cytokines, such as TNF, IL6, leptin, visfatin, resistin, angiotensin II and plasminogen activator inhibitor 1, whereas adipose tissue of lean individuals secretes anti-inflammatory adipokines such as adiponectin, as well as transforming growth factor (TGF), interleukin (IL) 10, IL4, IL13, IL1 receptor antagonist (IL1RA) and apelin. Polymorphisms in the genes of pro- and anti-inflammatory cytokines and/or their receptors may exacerbate cytokine imbalances and thus contribute to the development or worsening of obesity.

A wide range of pro and anti-inflammatory cytokines have been analyzed in relation to obesity, including some information regarding IL10.
locus analysis was performed with SNPassoc (version 1.9.2) considering four genetic models (codominant, dominant, recessive and overdominant). The

Statistical analysis was made using the SNPassoc package for R (version 1.9-2, R Foundation for Statistics Computing, https://cran.r-project.org).

4. Materials And Methods

4.1. Study subjects

All participants were recruited from the group of cadets of the Military University. Volunteers were acquainted with the protocol and research methods. The investigation protocols were performed in accordance with the rules of the World Medical Association Declaration of Helsinki, as well as ethical standards in sport and exercise science research. The procedures were accepted by the Ethics Committee of the Military Institute of Hygiene and Epidemiology - resolution number 07/2018 issued on February 23, 2018. Participants received a written information sheet concerning the study purpose, procedures used, benefits and risks, as well as a consent form.

The study enrolled 131 volunteers — male students aged 19–27 years old. Participants fulfilled the questionnaire screening for exclusion criteria including past diseases, injuries and the presence of severe and chronic pain of any organs or system, both in the past and currently. A general medical examination and electrocardiography (ECG) were performed to confirm the health condition. The cadets participating in the study constituted a homogeneous research group in terms of place of residence (they’re stationed on the university campus), nutrition (they were feeding at university canteen) and physical activity (they had similar levels of physical effort exposure due to their professional schedule).

Anthropometric measurements and body composition were obtained using standard methods. Height was measured using a portable stadiometer with a precision of 0.1 cm (TANITA HR-001, Tanita Corporation, Japan). Body composition and mass analysis measurements were performed using the TANITA MC-780 analyser (Tanita Corporation, Japan) according to the procedure specified in the instruction manual. The assessment of BMI values was made in accordance with the criteria set out by WHO (WHO 2000) [1].

The subjects were divided into two groups according to BMI value. The following formula, which is also applied in clinical practice, was used to obtain the BMI value: body mass index (BMI) = body weight/height² (kg/m²). The experimental group (OVERBMI) enrolled participants with BMI of ≥ 25.0, while the control group (CONBMI) consisted of men with BMI values between 20.0 and 25.0 [1]. For further analysis, an additional division was made according to the percentage of fat in total body weight (% fat). The control group (CONFAT) consisted of people with fat content below 20.0%, while the overweight group (OVERFAT) was characterized with fat content over 20.0% [36].

4.2. Genetic analyses

The buccal cell samples were collected with FLOQSwabs swabs (Copan Diagnostics Inc., USA). Genomic DNA was extracted using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany). The DNA extraction was performed according to the manufacturer's instructions. All samples were genotyped using TaqMan assays for IL10 (rs3024505) C___8828803_1, IL10 (rs1518111) C___12084302_20, IL10 (rs1878672) C__15983645_10, IL10 (rs3024496) C__15983681_20, IL10 (rs3024498) C__15983635_10, IL10 (rs3024505) C__15983681_20 single-nucleotide polymorphisms (SNPs) (Applied Biosystems, USA) on a CFX Connect Real-Time PCR Detection System (BioRad, USA).

4.3. Study subjects

Anthropometric data are shown as mean values ± standard deviation. Student’s t-test was used to determine the differences between the experimental groups. Statistical analysis was made using the SNPPassoc package for R (version 1.9-2, R Foundation for Statistics Computing, https://cran.r-project.org). Single locus analysis was performed with SNPPassoc (version 1.9.2) considering four genetic models (codominant, dominant, recessive and overdominant). The
models were constructed with respect to the minor allele. The statistical significant influence of single alleles on BMI and fat % was calculated using PearsonX² test with STAT package. Haplotype analysis was performed with the haplo.stats package. The haplo.score function was used to test the association, with magnitude and direction, between alleles combinations with BMI and with fat % under the different models of inheritance (additive, dominant and recessive). Odds ratio was calculated with haplo.cc statistic and haplotype frequencies with haplogroup function and genotype frequencies were analysed using Fisher's exact test. IBM SPSS Statistics (version 27) was used to calculate differences between groups with t-test. The level of statistical significance was set at the level of p < 0.05.

5. Conclusions

Obtained results indicate that IL10 polymorphisms are involved in the regulation of body weight in physically active people. Analysis of the association between IL10 rs3024505 and BMI showed significant correlation of this SNP with BMI value over 25. What is more, statistically significant association with BMI was found for CCGTA haplotype (rs1518111 C > T, rs1878672 G > C, rs3024496 A > G, rs3024498 T > C and rs3024505 G > A respectively). The obtained results extend and validate the theory that inflammation-related cytokine IL10 polymorphisms influence body weight parameters, with particularly emphasis on obesity, in physically active young men.

Declarations

Author Contributions: Conceptualization, E.M.; methodology, E.M., P.C., B.A. and O.A.; formal analysis, E.M., PR. and A.G.; investigation, E.M., B.A., O.A., E.S., A.B. (Anna Borecka) and P.C.; resources, B.A, O.A. and E.M.; data curation, E.M., P.C. and B.A.; writing—original draft preparation, E.M.; writing—review and editing, A.C., A.M., A.B. (Agnieszka Bialek), M.D.Z. and PR.; supervision E.M. and A.M.; project administration, E.M. and P.C. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Procedures used for this study were conducted in accordance with the World Medical Association’s Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Military Institute of Hygiene and Epidemiology—resolution number 07/2018, dated 23.02.2018.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Availability of Data and Materials: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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**Figure 1**

Schematic representation of SNPs in the *IL10* gene. SNPs positions with allelic variants (major allele>minor allele) followed by rs number are indicated. Open boxes indicate exons.