MULTIASPECTIVE DIAGNOSTICS OF TRAINING LOADS AS WELL AS BIOMECHANICAL PARAMETERS AND BLOOD INDICES AMONG LEADING ELITE RACE WALKERS PREPARING FOR PARTICIPATION IN THE OLYMPICS

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Abstracts:

Research problem: In the narrative review in the introductory chapter of this study, it was shown that there are no scientific methods or tools for researching the Functional Overreaching (FOR) state limit, which determines the occurrence of the phenomenon of Non-functional Overreaching (NFOR) and the overtraining syndrome – OTS.

Research objective: In principle, this research was conducted to provide evidence of the possibility of developing, implementing and controlling such a programme for preparation to participate in the Olympic Games of champions among athletes in race walking, which will guarantee that the applied training load does not cause a state of non-functional overload.

Material and methods: Six walkers took part in the research, presenting the level of the international and national master class at 20 and 50 km. They were all medallists of the Polish championship. Three of them have already participated in the Olympic Games, one was a medallist at the European and World Championships. In selected periods of the developed annual training plan, measurements in variability of walking speed at the level of the anaerobic threshold (starting speed) were carried out using the pitch test. The observation of the sports training process was supplemented with a multifaceted observation of the effects of overreaching training (ORT), which included the following measurements: 1. somatic features and anthropometric indices; 2. haematological parameters of the blood and non-enzymatic antioxidant factor, glutathione (GSH), vitamin D₃, and blood serum concentrations: pro-inflammatory cytokines -IL1β and IL-6, markers of oxidative stress, TAC, alpha- and gamma-TOC, proteins: Aponectin and Zonulin; 3. electromyographic (EMG) recording in the field and laboratory conditions and the work of the lower-limb muscles during the gait test with a gradual increase in its speed. Systematic monitoring of the nutritional status of walkers throughout the training cycle was performed and, on this basis, the following were established: a) principles of a rational diet and
Introduction

The vision for the development of competitive sport in all its disciplines is associated with the development of a talented athlete and bringing him/her to the championship class. The dynamics of sports results, and hence, the intensification of training loads, have exceeded the limits of human capability set by experts long ago. The ideal of the Olympic sport is to achieve a record result by practicing sports. However, this is very often done at the cost of losing health.

The essence of scientific research conducted in the field of sport is also acquiring new knowledge on the adaptation of the human body to extreme physical and mental effort and then, applying it to the needs of sports training. It plays an important role in bringing an athlete’s body to optimal performance (sports form) in a strictly defined time of competing at the highest rank of sports competitions. From antiquity, these include the Olympic Games. In a number of cases, however, the opposite effect is experienced during training. Training loads cause a state of decreased efficacy of the body as a result of implementing efforts at an intensity higher than the individual limit of functional overreaching (FO) allows. As it is known, this causes a favourable state of super-compensation and post-exercise adaptation. Physical efforts are a strong stimulus to induce beneficial skeletal muscle adaptations, starting with increased endurance due to mitochondrial biogenesis and angiogenesis, to the increased strength resulting from hypertrophy. This leads to exhaustion, which results in fatigue, which, in turn, causes metabolic drive that initiates more than moderate exercise activation of genes responsible for the remodeling of skeletal muscles [1,2]. While exercise is essential to induce and stimulate muscle adaptation, the recovery period after exercise is just as important. It should provide sufficient time for metabolic and structural adaptation of the skeletal muscles [3-5]. An imbalance between fatigue caused by intense training/competition and inadequate recovery periods after exercise/competition can lead to a decline in physical performance. Nonetheless, there is currently no scientific understanding of how long recovery periods should be for optimal skeletal muscle adaptation. Moreover, people who achieve the highest level of sports have a tight schedule of competitions, and therefore, have difficulty allocating time to recover between exercise sessions and competitions in which they should perform their maximum ability.

In fact, prolonged periods of imbalance between exercise load and body regeneration ultimately lead to extended periods of impaired starting performance [6-9] known as Non-Functional Overreaching (NFOR), which most often leads to Overtraining Syndrome (OTS). Such stages regarding the development of adverse post-training changes were defined only in the first decade of the 21st century [10, 11]. The first definition of overtraining syndrome [OTS] was assigned the following meaning: “OTS is defined by persistent underperformance despite >2 months of recovery, joined with changes in mood and absence of symptoms/diagnosis of other possible causes of underperformance” [12,13].

Practical experience and already advanced scientific research have allowed to show that very often, such a state of failure (training effect inversely proportional to workload) occurs most often in top-class athletes before and during major competitions [14-16]. Although its epidemiology is not large on a global scale, it certainly causes disappointment and discontent for hundreds of people practicing competitive sports at the highest world level. Overtraining may be the cause of a disappointment with unfulfilled aspirations to achieve record results after many years of physical and mental strain during sports training. This is sometimes the reason for ending a sports career.

According to reports from the previous century, at the 26th summer Olympics in Atlanta, the state of training overload was found in 28% of competing Olympians from different countries [17]. Overtraining also occurred in 10% of American athletes at the 18th Olympic Winter Games in Nagano [18]. Estimated data demonstrated that 7-20% of over-trained athletes can take part in the most important competitions worldwide [19]. In one
study, it was found that the state of non-functional over- 
load (NFOR) occurred at least once in a sports career amon
g ~60% of elite male and female runners and in 33% of lower ranked joggers [20]. From multi-centre, 
multi-national research, it resulted that 35% young swimm
ers starting their sports career were “over-trained” at 
least once [21]. This condition was found in 15% of elite 
English swimmers [22]. There are no statistics on the 
phenomenon of overtraining in Polish Olympians. 

The signalled problem turned out to be so interesting 
to solve in a cognitive and applicative sense in scient
ic research, that already in the last decade of the 20th 
century, discussion panels of scientists from the round 
table were organised in Oxford (Green College, March 
23, 1990 [23] and at St. Catherine’s College, April 19, 
1999 [24]), the topic of which was the issue of over-
training syndrome (OTS) among athletes. However, the 
greatest importance on the unification of positions on 
the definition of the 3 stages of training overload and the 
summary of research achievements as well as setting 
new directions for research on overtraining syndrome 
should be attributed to the initiative of the European Col-
lege of Sports Sciences [25-27], which conducted an 
ternational discussion with significant participation of 
American scientists to solve the indicated problem. This 
resulted in the development (after a critical analysis of 
research results from the turn of the 20th and 21st centu-
ries, of a common position on several key issues [28], 
aimed at solving such issues as: 
• the definition of Overtraining Syndrome (OTS) and 
the limits of reaching it through the already men-
tioned stages of overloading the body among the ex-
ercising persons: the stage of Functional Overreach-
ing (FO) and Non-functional Overreaching (NFOR); 
• the value of Overtraining Syndrome (OTS) hypoth-
eses: Glycogen hypothesis; Glutamine hypothesis; 
Central fatigue hypothesis; Oxidative stress hypoth-
esis; Autonomic nervous system hypothesis; Hypo-
thalamic hypothesis; Cytokine hypothesis, 
• proceedings in testing and diagnosing the limits of 
body overload among training athletes, 
• treatment of overtraining, 
• preventing the occurrence of Overtraining Syndrome 
(OTS). 

In each of the above-mentioned elements regarding 
the problems to be solved, and especially in the case of 
verification concerning the hypotheses of OTS forma-
tion, a reference can be found in the concept proposed 
by Edwards et al. from 1977 [29,30] regarding the ap-
pearance of Prolonged Low-Frequency Force Depres-
sion (PLFFD) in sports training, which is defined as: 
“a persistent exercise-induced reduction in submaximal 
force that can last for several days or weeks during the 
post-exercise recovery period” [31-34]. It was also 
noted that exercise (training) is not necessarily the only 
determinant of OTS occurrence, which is clearly empha-
sized by the use of the term syndrome to the word over-
training [28]. Both central (psychological, neurological) 
and peripheral (intramuscular) mechanisms have been 
assigned an important role in the development of OTS 
[27,28,35-37]. In such a multi-component etiology of 
the phenomenon, a complex set of psychological fac-
tors may be of equal importance, including excessive 
expectations on the part of the trainer/coach or family 
members, competition stress, personality structure, so-
cial environment, relationships with family and friends, 
training monotony, personal or emotional problems and 
school or work requirements. 

This is a significant statement in the direction of fur-
ther research on the cognitive and applicative aspects 
of the phenomenon of overtraining. First of all, in un-
derstanding the aetiology and prevention of overtraining 
syndrome, a multi-faceted approach should be used in 
the training process. Thus, no single marker can be tak-
en as an indicator of impending overtraining syndrome 
or non-functional overload [27,28]. 

In the common (anecdotal) meaning, however, the 
basic concept of overtraining is simple. It comes down 
to making pedagogical mistakes in the training process 
by using inadequate load stimuli to the tolerance of an 
athlete’s body. The exact cause of the syndrome is omit-
ted. In the scientific approach, without denying the im-
portance of such a causative factor, the sources of OTS 
are considered primarily in the pathological plexus of the 
disturbance in the functioning of the following param-
eters: physiological, immunological, psychological and 
motor [38]. Overtraining syndrome includes a systemic 
inflammatory process with diffuse influence on the neu-
rohormonal axis, affecting the athlete’s immunology and 
mood [28]. Unfortunately, it can be explored after fate. 
There are no objective methods of predicting its occur-
rence. So far, there are limited methods of pharmaco-
logical treatment [27,30]. 

Effective OTS prophylaxis is therefore the careful 
development of training programmes, which include 
regular monitoring by coaches and athletes themselves 
concerning the effectiveness of the training adaptation 
process (fitness) in the short- and long-term [24-30]. In 
addition, it should be supplemented with multi-faceted 
medical, physiological and biochemical research and 
nutritional status adequate to needs. 

Such assumptions became an inspiration to under-
take research on the best Polish race walkers from aca-
demic sports clubs competing in 20- and 50-km sports 
walking competitions. This is a sports discipline sport 
that allowed the Poles to win Olympic sport medals 
[39]). Hence, taking care and control of their preparation 
for participation in the 2017 Olympic Games in Rio de
According to previous research and our own observations, an interdisciplinary approach to the analysis of the race-walking technique, which seeks interactions at particular levels of the musculoskeletal system, plays an increasingly leading role [84-87]. While such studies provide detailed quantitative data, their applicative value is limited as they are conducted under laboratory conditions. There are no studies in which the movement technique of competitors at various competitions would be characterised, notwithstanding race-walking implementing precise measurement tools in field conditions. In our own research, such a complicated task was undertaken.

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According to the assumption, controlling the correctness of the training process and the race-walking technique of the tested candidates for the Polish national team was supplemented by the monitoring of their nutritional status, examining their health status and observ-
ing the status of haematological markers and those of biochemical oxidative stress, cytokines and hormones.

According to the assumptions, assessment of training process correctness and control of the race-walking technique of the tested candidates for the Polish representation for the Olympic Games was supplemented by monitoring their nutritional status and screening tests, including a comprehensive metabolic panel, complete morphology, which was supposed to exclude or confirm the existence of organic diseases that are the cause of worse results and are often associated with overtraining [25-27].

A separate group consisted of specialised tests: blood biochemical haematological markers and markers of Azonulin biomarkers, oxidative stress, cytokines and hormones, i.e. adiponectin, the indices of which were obtained during the sports training cycle and after the start for the 20-km race-walk. According to the position of the European College of Sport Science [25-27] and the American College of Sports Medicine [28], exceeding the range of reference values of indices subjected to exercise influence may be a signal of non-functional training overload or even an overtraining syndrome.

Research objective
Ensuring control over the implementation of the sports training process, the aim of which was to achieve by the best race walkers from the Academic Sports Association, the level of international championship in walking the 50/20-km distance, guaranteeing qualification for participation on the Polish national team at the Olympic Games.

Research issues
- What model of sports training was implemented in the annual training cycle?
- How was the training intensity of the best Polish race-walkers individualised in the annual training cycle?
- Why was the assessment of the race walking technique included among the training indices and how was its correctness tested in laboratory and field conditions?
- What was the health condition of the candidates for the Polish representation in the Olympic Games?
- What model of supporting the training process was used in the studied athletes through diagnosis, pedagogisation and recommendation of the proper nutrition process?
- Which of the considered haematological and biochemical markers were the most susceptible to the influence of exercise load and during sports competitions?
- To what extent could hormonal and protein markers be useful in controlling the limit of functional and non-functional overload and overtraining syndrome under the influence of the applied exercise loading race walkers?

Hypotheses
1. Experience of the coaching staff of a group of Polish race-walkers from the Academic Sports Association and the theoretical foundations of the modern sports training system will allow to develop a concept of their sports training, guaranteeing the achievement of sports results at the highest international level.
2. Optimising the implementation of training assumptions will be ensured by determining the ranges of individual training intensity on the basis of physiological field tests and the application of results regarding health control, nutrition, gait technique as well as biochemical, hormonal and cytokine haematological markers, based on the following premises:
   - determining speed and threshold heart rate in the physiological test allows to accurately and reliably establish an optimal training load to estimate the race-walking results for the 20- and 50-km race,
   - implementation of individual training loads, including those imposed on walking speed, will be guaranteed by control of the volume and intensity of each training unit by recording heart rate on the Sport Tester,
   - training control of health condition will allow for periodic elimination of ill athletes and their specialist treatment.
   - disturbances in the recording of bioelectric potentials regarding muscle work under training and competition conditions may be a signal for the occurrence of non-functional overload and the formation of Prolonged Low-Frequency Force Depression (PLFFD),
   - assessment of lower limb work on the basis of kinematic and dynamic analysis may indicate the source of disproportions in terms of muscle involvement in race-walking, which will allow training to be focused on strengthening those muscle groups that have been weakened by compensation and thus, prevent overloading and achieving sports results, disproportionate to the workload.
   - Observations and interviews indicate the prevalence of quantitative and qualitative nutritional mistakes, lowering the health and exercise capacity of various groups of athletes.
   - Modification of the nutrition model may contribute to the elimination of nutritional errors, increasing the effectiveness of training and optimal physical preparation as well as achieving top sports form.
   - Observations from coaching practice, recorded for many years, supported by the results of scientific research and being in accordance with the current state of medical knowledge, will allow for the de-
development of solutions, the detection of not only the problem of the motor apparatus disorders of the leading race-walkers (and their prevention), but also their functions of the immune system in the area of susceptibility to infections, inflammation.

• in addition to the obvious benefits in sports competition, the assessment of haematological, biochemical, hormonal and cytokine markers will primarily contribute to protecting the health of athletes and preventing the implementation of training loads leading to overtraining syndrome.

**Materials and methods**

**Subjects**

Six athletes practicing the race-walking competition participated in the study. The level of the assessed athletes: 4 subjects presented the level of the international championship class (GS, JJ, At, RS), 1 person the level of the M class (PG) and 1, the level of the class I (JH) (Table 1). All the competitors were medallists of the Polish championship. Among the competitors there were: participants of the Olympic Games, World Championships (GS, RS, JJ), medallist of the European Championships (GS).

**Scope, methods and research tools**

The material and time structure of the sports training among race-walkers in the Olympic macrocycle

The concept of the material structure was developed in accordance with the assumptions of the Polish methodology of training for walkers [40-47,88-92] distinguishing basic training measures, such as: General Race-walking Endurance in the Intensity Range: OWCH1, OWCH2; Pace Endurance in the Intensity Range: WT1, WT2, WT3; Special Endurance (WS); Relative Speed (Srel); Maximal Speed (Smax); Technique Rhythm (R); Walking Strength (S); Technique and Fitness Exercises (SPR) Main Losses and Race-walking Test (START); Continuous Running (BC).

The time structure of training of individual players was based on the guidelines of the Polish system of training in competitive sports [48-50,93] and the English names of its components were taken from the studies of American training periodisation [94,95].

**Selection of training loads**

Selection of training loads

The volume and intensity of basic training measures was individualised. In each mesocycle of the preparatory period, the basis for the application of the training load intensity in basic training measures (OWCH1, OWCH2, WT1, WT2, WT3; WS) (Tab. 2) was the result of determining the training and starting speed at the level of anaerobic threshold in the running exercise test carried out in field conditions [96,97], which was adapted to the needs of race-walking [98,99].

**Description of the test**

The test consisted of 5 efforts of varying intensity, continued for 6 minutes, with an interval of 2 minutes, during which the level of lactate in the blood plasma was measured. During each trial, the heart rate of the walker was measured on a Sport Tester with the Polar RS 400 Sport Tester. Its value was determined on the basis of averaged measurements from the last 3 minutes of exercise. It was found that in the first graded exercise, a walker's individual heart rate should be 50 bpm lower than the maximal heart rate (HRmax). In subsequent exercise tests, the heart rate increased by 10 bpm. During each 2-minute interval, the concentration of lactate in the blood of the walker was measured. For this purpose, the Lactate Scout device by SensLab GmbH was used. An increase of 0.5 mmol/L was considered the lactate threshold (LT).

**Tab. 1. Sports level and training experience of athletes under study**

<table>
<thead>
<tr>
<th>Sports level/sports class</th>
<th>At</th>
<th>GS</th>
<th>HJ</th>
<th>GP</th>
<th>RS</th>
<th>JJ</th>
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<tbody>
<tr>
<td>Training experience [yrs]</td>
<td>12</td>
<td>20</td>
<td>9</td>
<td>9</td>
<td>13</td>
<td>13</td>
</tr>
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</table>
The average speed to be reached was calculated on the basis of the length of the distance covered in the next trial in race-walking. The reference point for determining the intensity of walking in training and the predicted result in sports competitions was the average walking speed obtained in the exercise test, in which, based on the analysis of lactate in the walker’s blood, the level of lactate threshold was determined [98, 99].

In order to determine the maximal heart rate (HR\textsubscript{max}), maximal speed (V\textsubscript{max}) and lactate level (La\textsubscript{max}), measurement of the above-mentioned parameters was conducted after the completion of an additional 800-m walking test. The obtained results were recorded using Polar Pro Trainer 5 software. After this effort, the lactate concentration was measured during the 1\textsuperscript{st} and 3\textsuperscript{rd} minute.

**Testing sports walking techniques**

**EMG registration**

In order to assess the correctness of the gait technique, the work of selected lower-limb muscles was measured using kinesiological EMG during 6 physical efforts included in the previously described exercise test. This was done to determine the individual training intensity of the start [96,97]. The tests were carried out with the use of the NoraxonTeleMyo DTS wireless EMG measurement system. 6. The system complies with the standards of Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles and the International Society of Electrophysiology and Kinesiology (SENIAM and ISEK) for EMG measurement.

The placement and location of the electrodes were according to the recommendations by SENIAM (Surface EMG for Non-Invasive Assessment of Muscles) [100]. Therefore, 2 electrodes were carefully placed on the belly of each muscle, parallel to the muscle fibres with an inter-electrode distance of 20 mm. Due to the limited number of channels, we observed only the right limb muscles. The choice of these muscles was due to their involvement in the support and propulsion phase of the limb while walking. We registered the activity of the following lower limb muscles: Gastrocnemius Medialis (GM), Gastrocnemius Lateralis (GL), Biceps Femoris (BF), Tibialis Anterior (TA), Vastus Lateralis (VL) and Tensor Fascia Latae (T) and the lumbar muscle. The electrodes were not moved during the test.

**EMG analysis**

The raw EMG signal was processed using Noraxon’sMyoresearch software (MR-XP 1.07. Master Edition) in order to identify changes in amplitude during the whole test. The raw EMG data was filtered using a 50-Hz notch filter to remove any electrical interference from external sources. The signal was then filtered a second time using a 15-500 Hz band pass filter. This allowed noise or movement interference below 15 Hz and other non-physiological signals above 500 Hz to be removed. The data were smoothed using root mean squared analysis (RMS), which was calculated for a 50-ms window. To normalise our data, we used the Maximal Voluntary Contraction (MVC) recorded for each muscle prior to the test. This test normalisation makes the average EMG patterns highly reproducible and enables test comparisons.

For the analysis we used both: Mean [\mu V] and Relative [%] values of each muscle's EMG signal. Each registration was divided into 10-second periods and then, the amplitude was averaged in these intervals. Simple linear regressions were used to obtain coefficients of the slope.

It was assumed that the measurement of the lower limb muscle activity during the test described above will allow to notice the fatigue processes on the basis of the obtained electromyogram record and thus, disturbances in the correct gait technique [101].

**Testing nutrition state**

**Research methods**

In testing nutritional status of the race-walkers preparing for qualifying in the Olympic Games, a triangulation of qualitative and quantitative methods was used, namely observation and interview, using the sports nutrition monitoring technique as a tool.

The athletes’ nutrition was measured over a randomly selected period of 3 days prior to conducting the interview.

Analysis of energy consumption and basic nutrients as well as vitamins and minerals in food rations was carried out with the use of a computer nutrition program.

Evaluating the state of revitalisation was supplemented with an analysis of body composition.

**Organisation of research**

- During the training consultations, in the first stage of the research, interviews were carried out to diagnose selected parameters of the nutritional status and anthropometric measurements allowing to determine body composition and, indirectly, the manner of nutrition.
- A series of trainings was carried out on: the principles of a rational diet, nutritional recommendations when the body is loaded with physical exercise, dietary recommendations during the training period and the correct principles of post-exercise biological regeneration.
- Individual dietary consultations were carried out at subsequent meetings to eliminate errors and rationalise the sports nutrition model.

**Development of research results**

- In order to optimise the indices of the nutritional status and exercise capacity, the directions of di
etary modification aimed at the full implementation of quantitative and qualitative recommendations for athletes were defined.

- The reference points for the assessment of the subjects’ nutrition were the norms for the Polish population (RDA for people with high physical activity) [102,103] and quantitative recommendations for competitive athletes [104].
- Qualitative assessment of the athlete’s nutrition during the competition period included an analysis of the athletes’ food rations in relation to the quantitative and qualitative recommendations of the Swiss sports nutrition pyramid.

The results of the qualitative evaluation of the athlete’s nutrition were based on the guidelines of the quantitative and qualitative recommendations of the Swiss nutrition pyramid for athletes, which took the following criteria into account:

- insufficient consumption of vegetables (1 serving/day, on average);
- insufficient fruit consumption (0.8 portions/day, on average);
- partially insufficient consumption of whole grains and/or legumes (after adjusting for training) (3.59 servings/day, on average);
- definitely too low consumption of milk and dairy products (1.43 servings/day, on average);
- excessive consumption of non-recommended products (sweet and salty snacks as well as sweetened and alcoholic beverages) (1.27 servings/day, on average).

- Individual strategies of supplementing the athletes’ diets have been developed, contributing to the support of the body’s immunity.
- Athletes were prepared for consuming ready-made supplements in order to accelerate the process of physiological adaptation and regeneration following fatigue in the phase of functional training overload.
- Body composition was measured using the BIA (bioelectrical impedance analysis) method using the following indices: body fat, lean body mass, muscle mass, cell mass and water content.
- Measurements of body composition were carried out using BIA, using the AKERN Srl apparatus and the "Bodygram" computer program. In this way, the degree of organ fatness (percentage of body fat, body mass index (BMI)), water content in the body, muscle mass, cell mass and lean body mass (LBM), energy expenditure (adjusted to the proper body mass and recommended caloric value) was determined.

Haematological blood tests

Research methods

Blood before and after the walking trial, which concerned only hematologic data – measurement were performed using HORIBA ABX Micros 60 Hematology Analyzer device.

Blood collection and testing

Blood was collected from the ulnar vein without an anticoagulant, which, after clotting, was centrifuged at 3,000 RPM, serum was collected for determinations. The blood intended for plasma testing was collected into Vacuotainer tubes with EDTA K3, centrifuged for 15 minutes at -3,000 RPM, at the temperature of +4.0°C, plasma was collected from above the cell pellet. The serum obtained from whole blood, if measurements were not started immediately after centrifugation from the clot, were stored for up to 1 month at -20°C, if longer, then at -60°C. The storage was intended leave the serum in order to collect more samples from the tested subjects.

Blood morphology

Blood was collected during the interval between training sessions. The second blood sample was collected after the intensive race-walking.

The collected blood was examined for: the number of red blood cells, RBC (106/mm³), haematocrit: Ht (%), haemoglobin: Hb (g/dl), the average mass of haemoglobin in the blood cells: MCH, (pg), mean corpuscular volume, MCV (μm³), average concentration of haemoglobin in blood cells: MCHC (g/dl), the number of white blood cells: WBC (109/mm³), the number of platelets: PLT (1012/mm³). Measurements were performed using the HORIBA ABX Micros 60 Hematology Analyzer device. The number of reticulocytes was determined in relative terms in ‰. Our haematological research did not include a control group, therefore, thenormal data (concerns mean age 30-40 age) were compared to that obtained by: Lewis et al. [105], Kaushansky et al. [106].

Biochemical blood tests

Blood and serum collection as in haematological tests.

IL-1β

The IL-18 polypeptide was marked in the serum via the ELISA method, according to the strictly followed methodology of the DRG®IL-1β ELISA kit (catalogue item EIA-4437).

Interleukin IL-6

The Interleukin IL-6 polypeptide was marked using the ELISA method, according to the strictly followed methodology of the DRG®IL-6 ELISA kit (catalogue item EIA-4640), based on the Enzyme Amplified Sensitive Immunoassay performed on a microtiterplate.

Total antioxidative status/capacity (TOC/TAC) in the serum

The total oxidative capacity (TOC) and the total antioxidative capacity (TAC) reactions are based on the speed of the distinct antioxidants in the samples. The measured concentrations of antioxidants are equivalent to the re-
activity of the distinct antioxidants and not to their total amount in the samples. Therefore, the test system H$_2$O$_2$ equivalents must be used in this estimation as the unit for antioxidative capacity.

The methodology of preparation of antioxidant (TOC/TAC) determination was based on a strictly defined procedure according to the LDN kit, catalogue item TOC LDN Test, item category DM P- 4200, for TAC: DM P- 4100.

Procedure for preparation of serum for testing – as in the case of IL-6 ELISA. Samples with lipaemia and traces of haemolysis were not included in the study.

**GSH reduced glutathione**
The level of reduced glutathione was tested in venous whole blood via the spectrophotometric method using a 412-nm wave, according to the Beutler method [107]. Glutathione was determined immediately after venous blood sampling.

**Adiponectin 30-kDa**
The protein in the serum of the athletes was tested via ELISA, a test prepared for adiponektin E09 using 2 specific and highlyaffineantibodies.

The determination procedure was carried out strictly according to the guidelines of the KIT – Adiponektin ELISA kit, item HE09 by Mediagnost®.

**Zonulin**
The level of zonulin was determined in the plasma according to the guidelines included in the DRG® Zonulin Serum ELISA kit, cat. pos. EIA - 5515.

**25-OH Vitamin D (total) ELISA**
Vitamin D$_3$ was determined in the athletes’ plasma with a test based on the enzyme immunoassay measurement totalling 25-OH Vitamin D, named vitamin D$_3$.

The determination procedure was followed strictly according to the DRG® regulation, 25-OH Vitamin D (total) ELISA, cat. pos. EIA-5396.

**Research Results**
General characteristics of the time and material structure of sports training, taking into account the volume and intensity of training carried out by the 6 tested race-walkers in the annual Olympic training cycle.

**Training time structure**
In Fig. 1, an example is presented regarding the development of the training organisation scheme for a competitor appointed to the Olympic team of race-walkers from the Polish Athletics Association (PZLA)
In Fig. 2, a diagram is presented of the construction of the annual training cycle for 6 athletes from the Academic Sports Association (AZS), implementing the author's training model in connection with the multifaceted observation of the effectiveness regarding the process of functional training overload. In accordance with the adopted assumption, the training load of race-walkers planned for use 365 days a year, was divided into tasks carried out in 51 weekly microcycles and 7 mesocycles of sports training. In accordance with the adopted scope of the research and its purpose, the scheduled dates of performance in the mesocycles: preparatory and specific sub-phases (BPS) were planned.

Assumptions for planning training in Direct Start Preparation (DSP)

In developing the concept of individual training plans for individual athletes in specific sub-phases (BPS), the developed scheme of organisation and training load was taken into account, developed on the basis of the multi-criteria decision support method of the Analytical Hierarchical Process (AHP) created by T. Saaty [92].

Physical structure of training

**General volume and its components**

In Table 2, the statistical characteristics are presented of the realised training volume (km) in the annual training cycle, with the division into the names of the training measures used.

Analysis of the presented training volume in sports walking, which was carried out by the athletes from the Academic Sports Association in the annual cycle of sports training, was within the lower ranges of the volumetric load variability implemented by the best race-walkers in the world during periods when they set their personal best records for 50 km. According to data from 2017 [92], the level of the international master class was achieved by training with a volume between 3,001-6,894 km. The upper ranges of variability for the training volume of the studied athletes were also similar to the load ensuring success for the Polish world record holder and Olympic champion for 50 km – RK [89,92]. On this basis, it can be assumed that the race-walkers included in the cycle of organised preparation for the start at the Olympics followed the Polish model of training, in which more intensive training measures had a high preference. Thanks to them, Polish walkers obtained comparable sports results to those achieved by the world elite of race-walkers who carried out training with a much larger volume. In some cases, the talent of the best Polish walkers allowed them to reach an even higher world level.

### Tab. 2. Arithmetic means and range of training volume variability (km) of basic components of the material structure of training in the annual mesocycle of training walkers

<table>
<thead>
<tr>
<th>Training volume [km]</th>
<th>Total</th>
<th>BC</th>
<th>OWCH₁</th>
<th>OWCH₂</th>
<th>WT₁</th>
<th>WT₂</th>
<th>WT₃</th>
<th>WS</th>
<th>SCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td>3866</td>
<td>106</td>
<td>3038</td>
<td>373</td>
<td>71</td>
<td>72</td>
<td>50</td>
<td>83</td>
<td>73</td>
</tr>
<tr>
<td>min</td>
<td>3449</td>
<td>90</td>
<td>3093</td>
<td>155</td>
<td>0</td>
<td>69</td>
<td>0</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>max</td>
<td>4181</td>
<td>112</td>
<td>3210</td>
<td>407</td>
<td>85</td>
<td>99</td>
<td>65</td>
<td>109</td>
<td>94</td>
</tr>
</tbody>
</table>

**source**: own research
Training intensity

In Table 3, the reference points are presented for determining the training intensity of the tested athletes, such as: heart rate, walking speed and the level of lactate found at the lactate threshold in the conducted stress test.

Based on the analysis of the presented data, it can be concluded that at the level of the anaerobic threshold, the mean heart rate in the subjects was 160 bpm (SD 8.13), which was 87% of its maximum values (HR max), the mean level of lactate in the blood plasma was 2.2 mmol (SD 0.50), with a clear range of variation: 1.5–3.0 mmol.

At the lactate threshold level, the subjects covered the test distances with an average speed of 3.6 m/s (SD 0.19). This is a very important indicator. Such speed at the level of the threshold and the frequency of heart contractions is already widely regarded as the reference point for determining the intensity of the exercise load in the physical structure of training which was presented earlier [93,99]. It is also theoretically possible to relate the value of the threshold velocity to the possibility of obtaining a result in 50- and 20-km walking distances by each of the participants, assuming a 4% loss of such speed in walking for 50 km and the possibility of continuing efforts in walking for 20 km at a speed higher by 4% [98]. Therefore, assuming that in the mesocycle of direct preparation the athlete had a threshold speed of 3.78 m/s, s/he could theoretically obtain a result of 3 h 42 min in the 50-km race-walking competition, which would be close to his/her personal best. From the calculation of the results for the entire group (at an average speed of 3.56 m/s), theoretically, it would be possible to determine the achievable result for 50 km at the level of 3 h 49 min. After rejecting the 2 lowest indices of threshold speed, i.e. 3.33 m/s – their result for 50 km = 4 h and 38 min; the next 3 athletes would have a similar chance to achieve a place in the Olympic representation.

The relatively high level of training in direct preparation in the qualifying competition for a place on the Olympic team may be demonstrated by the dynamics of speed increase in subsequent trials of the stress test.
until reaching the threshold speed (Tab. 4) and the value of the maximal speed obtained in walking in the last test section of the 800-m distance (finishing speed), as well as the rate of lactate restitution level in the 1st and 3rd minutes following completion of the final test trial (Tab. 5).

The purpose of introducing the 800-m stress test, carried out at a maximal walking speed, was to determine the maximal heart rate of the subjects and the finishing speed, which is important for determining the intensity of the training load above the anaerobic threshold [93]. On the other hand, the measurement of restitution at 1 and 3 minutes after exercise is considered a training index [110,111]. As shown in the data presented in Table 5, the mean maximal heart rate in the subjects was 184 bpm (SD=5.05), mean speed of 4.32 m/s (SD=0.35) for the 800-m distance. The highest speed of 4.6 m/s was achieved by JJ, and the lowest by HJ: 3.65 m/s (d=1 m/s). The highest lactate level in the 1st minute following exercise was 12.7 mmol/l. This was found in HJ. The arithmetic mean in all examined walkers was 10.2 mmol/l (+/- 2.29). During the 3rd minute of recovery, there was a decrease in lactate levels to an average level of 7.6 mmol/l (+/- 1.69). It should be emphasized that in all subjects, the lactate level was quickly restored 3 minutes after exercise. On average, this totalled 70% compared to the test carried out in the 1st min.

Such a high training index could be achieved by supporting sports training with the results of multifaceted research aimed at controlling the correctness of the course of functional overload among athletes and supporting the processes of post-exercise regeneration of intensively training race-walkers. This is considered the basic condition for counteracting the undesirable phenomenon of overtraining syndrome [25-28].

Electromyography

Comparison of individual muscles responses, depending on the intensity of walking, was described by the slopes of the regression lines. To illustrate individual responses of the muscles, we have provided selected scatter plots below. Since our observation revealed different responses of the muscles in our subjects: in some, an increase of the EMG activity peak in the course of fatigue was noted, whereas in others—a decrease. That is why we decided to provide the results for 1 of the athletes (GS) (see below).

Based on the conducted registration, the changes in the peak value of the EMG amplitude turned out to be relatively small. Also, signal normalisation did not significantly change the results. In some cases, the tested muscles showed an increase in peak activity during exercise, while in others, a decrease was noted.

The gaps in the figures (TA, GM) followed from the technical difficulties that we faced during the process of recording (sweating skin due to experiment performed in dynamic conditions made it impossible to obtain a signal throughout the whole experiment).
Therefore, on their basis, it is difficult to decide whether changes in electric potential indicate the phenomenon of muscle fatigue or the reorganisation of muscle activity due to individual adaptation to long-term repetitive movement (walking).

**Monitoring nutrition**

a) The assessment of energy consumption and basic nutrients in the average CRP among the group of athletes showed incomplete balance of food rations in terms of the share of macronutrients, particularly including:

− energy consumption close to the norm (about 3,000 kcal/day);
− share of protein in the CRP energy pool close to the norm (17.15% of energy);
− proper protein intake (in g per kg body mass) (1.76 g/kg bm)
The assessment of consuming selected vitamins including:

- insufficient share of total carbohydrates in the CRP energy pool (50.94% of energy);
- low consumption of total carbohydrates (in g per kg body mass) (5.67 g/kg bm);
- low consumption of digestible carbohydrates (in g per kg of body mass) (5.31 g/kg bm);
- slightly exceeded (by 0.75%) share of sucrose in the CRP energy pool (10.75% of energy);
- consumption of fibre within the lower range of the norm (26 g/day);
- slightly exceeded share of fats in the CRP energy pool (31.92% of energy);
- high total fat consumption (in g per kg of body mass) in relation to the norms for the Polish population and remaining within the upper limit of the Celejowa norm (1.49 g/kg bm);
- excessive share of saturated fatty acids in the CRP energy pool (11.12% of energy);
- excessive consumption of cholesterol (423.21 mg/day).

b) The assessment of consuming selected vitamins in the average CRP of the group of competitors showed incomplete balance of food rations in terms of the content of certain ingredients, particularly including:

- excessive consumption of vitamin A in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (1351.29 μg/day);
- high vitamin D deficit in relation to the adopted nutritional norms (min 3.80 μg/day);
- excessive consumption of vitamin E in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (max 13.41 mg/day);
- excessive consumption of vitamin B1 in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (1.84 mg/day);
- excessive consumption of vitamin B2 in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (2.04 mg/day);
- excessive consumption of vitamin B6 in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (2.34 mg/day);
- excessive consumption of vitamin B12 in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (4.14 μg/day);
- excessive consumption of vitamin PP in relation to the norms for the Polish population, and close to the norm for athletes (27.91 mg/day);
- intake of folic acid at a level close to the lower limit of the norm for the Polish population (315.56 μg/day);
- vitamin C intake at a level close to the lower limit of the norm for the Polish population, and the deficiency in relation to the norms for athletes (78.74 mg/day).

c) Assessment of consuming selected minerals in the average CRP of the group of athletes showed incomplete balance of food rations in terms of the content of certain ingredients, including, in particular:

- calcium consumption close to the norm in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (888.37 mg/day);
- excessive consumption of phosphorus in relation to the norms for the Polish population, and within the norm for athletes (1.799.82 mg/day);
- excessive consumption of iron in relation to the norms for the Polish population, and deficiency in relation to standards for athletes (14.37 mg/day);
- magnesium intake within the norm in relation to the norms for the Polish population, and the deficiency in relation to the norms for athletes (372.21 mg/day);
- excessive zinc consumption in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (14.46 mg/day);
- excessive consumption of copper in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (1.42 mg/day);
- excessive iodine intake in relation to the standards for the Polish population, and deficiency in relation to the standards for athletes (178.34 μg/day);
- excessive sodium intake in relation to the norms for the Polish population, and deficiency in relation to the norm for athletes (4.644.41 mg/day);
- deficit of potassium intake in relation to accepted norms (3,692.46 mg/day).

d) The assessment of the degree of implementing the quantitative and qualitative nutritional recommendations of the Swiss Sports Nutrition Pyramid showed a limited scale of rational nutritional choices.

e) The nutritional and dietary errors identified in quantitative and qualitative research on the nutrition of athletes have become the basis for the development of directions for modification, i.e. rationalisation of the nutrition model in order to improve indicators of nutritional status and exercise capacity of athletes.
Haematological blood tests
Biochemical tests carried out in several series of measurements in this research allowed to indicate the correct course of adaptation to physical exercise.

The research material consisted of peripheral venous blood immediately before and after exercise. On the basis of sample No. 1, the blood counts were determined, analysed with the use of the ABX whole blood analyser, giving the counts for RBC erythrocytes (Hb, Htc, MCH, MCHC, MCV), leukocytes and platelets. Blood smears were performed to determine the percentage of leucocyte morphotic elements and the activity of neutrophil myeloperoxidase. The activity of this enzyme in individual leucocytes was calculated using a computer program based on microscopic densitometric analysis. Sample No. 2 was intended to obtain plasma after centrifugation of whole blood. Plasma was stored -70°C for comprehensive testing of interleukins, viral antibodies, myeloperoxidase, glucose, vitamin D3 and NO. Blood test No. 3 was whole blood used to determine the level of GSH depending on the material resources of other biochemical blood indices. The samples were stored at a temperature of -70°C. Analysing the obtained results of trial No.1, concerning the pre-exercise period and immediately after vigorous exercise, and in resting state, it was found that the data should be verified in 2 ways: 1. a detailed analysis of date considered individually for each athlete; 2. Averaging the obtained results and formulating conclusions regarding the legitimacy of performing such a vigorous effort.

The erythrocyte lineage data showed stability over the 3 study periods. Only a slight decrease in MCV-μm³ was observed, which caused attention to be paid to the correlation with the unchanged haematocrit number. As for the number of white blood cells, after exercise, they increased by an average of 2,000 per 1 mm³, which, compared to the data presented for the world population (5,000-6,000), was a puzzling value and perhaps indicated muscle damage during physical exercise.

The RBC showed a slight increase of 1 to 2 million in 1 mm³ after intense competition, returning to the norm at rest, to a value of 5.0 x 10¹² (l/μl).

The WBC count demonstrated an increase after vigorous exercise from 1,000 to 2,000 in 1 mm³, with an average value of 6.0 x 10⁹ (l/μl).

PLT increased after vigorous exercise by an average of 50,000 per mm³, with a mean at rest totalling 250 - x 10⁹ (l/μl).

Hb levels were remarkably stable with no difference in the rest/exercise ratio, ranging from 14-15.5 g/100 ml.

Following vigorous exercise, haematocrit decreased on average by 4.0%, compared to the mean within the range of 34–40%.

The indices: MCV 84 - 94 μm³, MCH 27 - 34 pg and MCHC 31 - 37 g/100 ml, were within physiological limits and did not fluctuate regardless of the state of rest or exercise intensity.

The average value of reticulocytes - 10 ‰, increased by 2 ‰ after exercise.

Biochemical blood tests

In Table 7, mean measurement values are presented for biochemical parameters which are significant in the control of the exercise load process and the state of functional overload. Their description and meaning are presented in more detail in the ‘Discussion’ section.

### Summary and Discussion

In accordance with the adopted research programme and the recommendations of the European College of Sport Sciences [25-27], as well as the American College of Sport Medicine [26,27], continuous monitoring of training loads, recording of the material and time structure of training, and monitoring the nutritional status of athletes...
the best Polish race-walkers from academic clubs were implemented. This was supplemented with a series of electromyographic measurements for the working muscles among athletes practicing race-walking in order to evaluate sports technique. In addition, in order to ensure the proper course of the adaptation process after functional overload, nutritional control and supplementation were applied adequate to needs. In order to exclude the state of non-functional overload or the onset of the overtraining syndrome, medical control of the subjects' health as well as biochemical and haematological blood testing, were carried out in the preparatory phase, in the period of immediate preparation for the start, before and after the control competition, which turned out to be useful.

The purpose of the developed model of sports training and the conducted multifaceted research have been reduced to the need of optimising sports training and achieving the Olympic index by those implementing it. This was the first stage of applying for promotion to the Polish national team at the Olympic Games in Rio de Janeiro. As already mentioned in the characteristics of the subjects, this was achieved by 1 competitor from the group covered by our team with coaching care and re-

The most significant cognitive achievement is the use of the model of the sports training system for race-walkers in the period of immediate preparation for the start, based on the assumptions of the Analytical Hierarchical Process, the creator of which was the eminent American scientist – Professor M.L. Saaty [92,112].

In terms of application, the high useful and prognostic value of the method for determining the optimal training and starting load in race-walking the 50- and 20-km distances, based on the speed obtained at the lactate threshold level during the stress test [96,97], which was modified (by the author) [98- 99] for the purposes of training and predicting performance in race-walking. The results of our own research confirmed earlier suggestions [98] that the effort at the threshold intensity may be continued for about 2.5 hours. At a distance of 20 km in race-walking, the athletes develop a speed higher than that of the threshold (104% of the speed set at the anaerobic threshold), and for 50 km, the walk is continued at a lower speed (96% of threshold speed). The physiological and metabolic adaptations that occur in response to training have been extensively investigated. Oxygen consumption, blood lactate threshold, heart rate intensity, respiratory exchange ratio and the pulmonary ventilation threshold are common measures used to reflect endurance performance or adaptation to training [93]. Thus, it should be noted that training and starting speed in long-distance race-walks, established at the level of anaerobic changes, has only recently entered the practice of sports training in Poland [98,99,113-116]. A review of literature allows to show that - so far - this method of intensity control has not found any imitators outside our country. The reasons for this are probably (apart from the costs of carrying out tests on expensive specialised equipment) certain safety considerations accompanying the collection and analysis of blood, i.e. tests conducted on humans. Such research must be carried out in accordance with established legal acts and generally accepted ethical standards, referred to as the Declaration of Helsinki, regulating clinical trials, as well as the Principles of Good Clinical Practice. The consent of the Bioethics Committee was granted to conduct this study research.

It is difficult to expect that the applied model of sports training and its support through scientific research could be widely popularised in training practice. It can be assumed that in the future, the results of our own research and the acquired practical experience may be used in scientific discussions on counteracting the occurrence of non-functional overload and overtraining syndrome. Certainly, a number of results obtained in this field of research have high cognitive value, which is it the bottom of popularisation and implementation into coaching practice. An example of this may be haematological and biochemical blood tests and the developed programme of nutrition and supplementation of athletes. Although our biomechanical analysis did not provide equivocal conclusions, the observation of interaction between the neural and muscle systems is fundamental to all movements.

**Haematological testing**

As mentioned earlier, the blood image was normal; including the parameters examined, both during the training period and after maximal exercise. Slight fluctuations concerned haematocrit for physiological reasons (commonly known) – post-exercise dehydration, which returned to the norm during the rest period (the next day), i.e. 30-40%. The unchanging value of the number of erythrocytes, correlated with the haematocrit value, indicated no physiopathological disturbances.

Haematocrit testing is an important element in exercise physiology, which must be carefully considered as an important indicator of possible water and electrolyte disturbances.
Regarding the blood count – the percentage relationship with particular focus on the granulocytic line, the results cannot be considered using the percentage averaging method, but only individually. In the case of this study, by assessing the results of blood counts and its biochemical data, one of the competitors detected allergic changes that were not characterised by symptoms described on average for this type of disease. Therefore, in this case, specialist allergological diagnostic tests and medical consultations were ordered, as a result of which the athlete (who had previously received only symptomatic medications, despite growing problems), started immunotherapy, i.e. causal treatment. After the haematological check-up, the competitor’s blood was subjected to the established tests.

Such an intervention is an example of the fulfilment of one of the assumed objectives of our research – covering the leading athletes with in-depth diagnostic care during the training and competition cycle enables the detection of possible health disorders and helps to increase or restore maximal fitness and prevent situations in which, despite strenuous efforts and increasing training loads, competitors fail to achieve expected sports goals. Only multiple blood tests treated individually indicate the physiological state of the athlete, while after averaging the data for several athletes, the statistical population results blur the individual image that is important for assessing the response of a given organism to various endogenous and exogenous loads during vigorous exercise.

In a publication devoted to research on marathon runners [117], the authors paid special attention to significant fluctuations in leukocyte levels among athletes before and after exercise, reaching values from 5,000 to 17,000 in 1 mm³, presenting their research results and citing 8 authors from specialists in this field of sports exercise research. It is worth noting that the race-walkers did not show such a large variation in the number of white blood cells as marathon runners after vigorous exercise.

An important result is the calculation of the reticulocyte per ml in the blood image, maintained within the physiological range, which proves the proper function of the marrow/blood barrier, despite the vigorous effort of the participants. The literature on haematological data of healthy men aged 30-40 years, obviously examined under resting conditions (fasting), was carefully analysed and the data obtained in this study were related to them. There was clearly minimal deviation after vigorous exercise in race-walkers [118-120].

The level of cytokines in the blood plasma was tested: interleukins IL-1β and IL-6 using the ELISA technique. Levels of IL-6 as myokine increased with exercise intensity, while levels of both IL-6 and IL-1β decreased during the resting period (but not for all athletes the following day). Thus, significant individual variation in the levels of both cytokines was observed. IL-1β, IL-6 and TNFα are among the most rapidly released cytokines in response to exercise stimuli [121]. Molecular mechanisms involved in the repair of damaged muscles include increased levels of IL-1, IL-6 and IL-10 [122]. IL-1β stimulates the synthesis of the BDNF (brain-derived neurotrophic growth factor), which regulates the proliferation, differentiation and survival of neuronal cells, and enhances the production of reactive oxidative and stress (ROS) involved in gene expression [123]. IL-6, on the other hand, is a multidirectional cytokine. It activates T lymphocytes, regulates the growth and differentiation of B lymphocytes, stimulates the release HSP (heat shock proteins) from the liver, the expression of which increases when cells are exposed to stress factors, e.g. high temperature or ROS [124]. IL-6 participates in energy management by regulating the level of glucose and free fatty acids (FFA) in the blood. In case of lipid economy, it was found that IL-6 stimulates lipolysis in adipose tissues, increases the concentration of triacylglycerols and FFA in the blood, and enhances β-oxidation of fatty acids in muscle cells [125]. In this study, no changes in the level of the tested cytokines were observed. However, the participation of the discussed cytokines in the coordination of inflammatory processes and regeneration during muscle reconstruction cannot be ruled out, as indicated by the study results [126]. This is confirmed by the negative correlation between TOC and IL-6 noted on the first study date. A high positive correlation was also demonstrated [127] between the markers of ROS activity and IL-6, which not only indicates the participation of ROS in the release of this cytokine into the blood, but also the possibility of modulating its level with antioxidants.

Research on the level of glutathione (GSH) – as an important antioxidant compound, showed dispersion of data that is difficult to interpret, especially in the period following vigorous exercise, but the search for relevant literature that could be helpful in this interpretation of the results attracted our attention in 2 publications [128,129]. Their authors present the selection of an appropriate research method for GSH, state that so far, global laboratories have used a commonly incorrect methodology affecting the level and dispersion of results. Also in our research, the commonly recommended methodology was used, with a certain error of GSH instability. According to the 2 new laboratories mentioned: (a) the blood must be tested – preferably – immediately; (b) some kind of antioxidant stabiliser must be used in the form of added N-ethyl maleimide at an appropriate concentration.

These tests should be an indication for laboratories...
Adiponectin

As it is well-known, adiponectin is a polypeptide produced by adipose tissue, acting as a protein hormone, participating in a number of important metabolic life changes. It actively participates in energy balance, which is especially important for athletes. It is associated with the regulation of intravascular pressure, glucose metabolism and fat oxidation. Adiponectin bound to adipose tissue secures the relationship of yellow marrow to red [132]. This polypeptide should be marked for health control in terms of the internal physiological balance of athletes, especially competitive ones. According to Kratzsch and Kiess [133], and Kratzsch [134], the average norms for healthy people range between 7 and 21 μg/ml. In our research, the level of adiponectin in resting state was 21.0 μg/ml, while after the competition, it totalled 18.8 μg/ml – a slight decrease that was not statistically significant. The stability is of great importance, taking the above-mentioned participation of this polypeptide in the metabolism of the body into account.

Zonulin

Zonulin is a parameter related to the permeability of the intestinal wall and is associated with the immune system of the GALT (gut associated lymphoid tissue) system. This system is based on the cooperation of specialised cell systems, such as: Peyer's patches, lymph nodes and free cells of T and B lymphocytes, macrophages, dendritic cells; the whole interaction participates in the synthesis of IgA, as a barrier to pathological bacteria and their toxins, an asymbiotic reaction with intestinal bacteria inhabited from birth [135]. It is an important indicator which, in the case of increased values in the tested individuals, may indicate disturbances in the intestinal function related, on the one hand, with poor absorption of nutrients and, on the other, with the risk of developing allergies or food intolerances. In the case of elevated plasma zonulin values, it is advisable to choose an appropriate diet and adjust the training method to increase an athlete's efficiency. This protein should be recommended as an indicator of the proper permeability of intestinal enterocytes, especially in athletes who often visit different cities, countries – with a varied diet that can significantly reduce the intended effects of a successful victory in the competition. The most adequate measurements of the zonulin level are carried out in the stool, where the average values of 60 (± 46) ng/ml in healthy individuals are given, while for the serum values, these are 10.0–48.0 ng/ml. In our research, it was shown that the remaining athletes demonstrated 8.15 ng/ml, and after exercise, a significant decrease to 6.1 ng/ml was noted. Interpretation of the reduction of zonulin after the competition is quite difficult, especially with the tendency of "post-exercise thickening" of plasma after significant sweating of the athletes.

Plasma levels of vitamin D3 (and data obtained from dietary studies in this project) indicated its deficiencies in all tested athletes and an additional reduction, especially after the most vigorous efforts in some of the participants. Supplementation and an appropriate diet (as a mandatory recommendation in the case of vigorous exercise) restored its normal levels, which was observed in some cases. The inverse correlation between the concentration of IL-6 and calcidiol, noted after the end of the training programme, shows that vigorous race-walking does not induce inflammation in working muscles. As shown by the results of studies by other authors [136], vitamin D deficiencies contribute to the loss of muscle mass and the reduction of its strength [137], which is associated with the reduction of vitamin D receptors (VDR) in the muscle tissue [138]. Calcidiol also has anti-inflammatory properties, and its deficiency is associated with increased inflammation. In the research by Schleitoff et al. [139], vitamin D, supplementation has been shown to reduce the release of pro-inflammatory factors such as CRP, TNF-α and IL-6.

In this study, a slight increase in the level of the tested vitamin was noted on the second day following...
an intense start, but it was not statistically significant, which can be considered a favourable phenomenon in the face of, e.g., a decrease in its level. This may prove beneficial stability of the vitamin, which is so important in the work of muscles. The correct level of this vitamin is within the range of 3.5-130 ng/ml. The fluctuations are closely related to age as well as the amount of sun present for a given territory.

More recent studies allow to indicate that one should be very careful about the possibility of achieving positive sports effects as a result of a significant increase in the dose of vitamin D, beyond the range of medical standards recommended for athletes [140].

Chronic vitamin E supplementation has been shown to worsen athletic performance and is not currently recommended for athletes [141]. In the most current studies available, no consistent positive effects have been shown for vitamin E supplementation on the health or athletic performance of most athletes [142,143]. It has been suggested [141] that supplementation with antioxidants will benefit athletes when performance adaptation is not the primary goal and immediate performance improvement is desired. A short-term positive effect of vitamin E supplementation in 2 areas of sports performance was found [144]. The first is in the case of alpine training. It was found that vitamin E supplementation showed a positive effect on athletes training at high altitudes by reducing red blood cell (RBC) deformation. However, the results are still inconclusive and warrant further research [141]. The second area of interest was ad-hoc supplementation before important competitions. Acute antioxidant supplementation has been shown to improve performance during high-intensity exercise with short intervals for recovery [145].

There are many studies on the effects of vitamin E supplementation in combination with vitamin C intake. Vitamin C is known to be an essential component of the diet and may reduce the adverse effects of exercise-induced reactive oxygen species, including muscle damage, immune system dysfunctions and fatigue [143,146]. While there are potential benefits of antioxidant supplementation in people undertaking exercise, some evidence suggests that supplementation with vitamin C and E may weaken rather than improve some acute and chronic adaptive responses to exercise [9,10,40,147-149]. It was found that continuous supplementation with high doses of antioxidants weakened some adaptive responses to resistance [10,50,146,148,150] and endurance training [9,40,147,148].

This phenomenon has been confirmed in more current studies [11,151]. In them, it has been shown that vitamin C in higher doses reduces training-induced adaptations by reducing biogenesis mitochondria or possibly, by altering vascular function (>1 g•d⁻¹). Therefore, athletes practicing race-walking were suggested to take small doses of vitamin C (0.2 g•d⁻¹) in 5 portions of fruit and vegetables per day, which should be enough to reduce oxidative stress and will not exceed the threshold that will disturb optimal training adaptation. Short-term consumption (1 to 2 weeks) of more vitamin C > 0.2 g per day was recommended for athletes during periods of increased training stress.

The problem of joint intake of vitamin C and E was taken into account in training and supplementation programmes. They are key components of the co-operating network of the antioxidant defence system [152]. Alike vitamin E as an antioxidant, vitamin C has the ability to provide protection against lipid peroxidation by scavenging free radicals. After all, it should also be considered that both vitamins C and E act synergistically [143,151], with vitamin E acting as the main antioxidant and the resulting vitamin E radical then reacting with vitamin C to regenerate vitamin E. The interaction between vitamins E and C is therefore based on the “recycling of vitamin E". Thanks to this, vitamin E, tocopherol, reacts with the peroxide radical to form the tocopheryl radical, which, in turn, is regenerated by vitamin C [153]. This recycling of vitamin E requires the supply of vitamin C. It was also the reason why both vitamins were included in the developed protocols of the diets and supplementation for the athletes under study. The race-walkers were recommended both nutrients be consumed simultaneously.

Taking the presented reservations into account about the supplementation and nutrition of vigorously training athletes and, at the same time, the need to satisfy the needs of training race-walkers with antioxidants, mainly vitamin E after starting in the 20- and 50-km race-walks, it was suggested they focus on consuming products rich in fruit, vegetables and other products that are not present in the produced supplements or are selected in single doses.

Conclusions

1. The results obtained for the tested athletes at international competitions allow to formulate the conclusion that the original model of training for race-walkers implemented by them, based on the experience gained in practice and the current knowledge on the adaptation of athletes to high training loads, implemented at the threshold of functional overload, can be considered a model for the method of preparing athletes for highest-ranking sports competitions in the world.

2. The possibility of safely implementing the proposed model of training loads was confirmed by the results of multifaceted control studies regarding the process of shaping race-walking technique, diet and variability of haematological and biochemical blood indices.
carried out during training in the preparatory period and in the period of safe preparation, before and after the start of athletes in international-class race-walking competitions.

3. Documentation of materials from the observation of the movement technique proved the usefulness of EMG signal recording using the TeleMyo DTS wireless device by Noraxon.

4. The recording and analysis of the EMG signal parameter values did not provide grounds for identifying disturbances in walking technique or the occurrence of the muscle fatigue phenomenon during training in environmental conditions of holding sports competitions.

5. The lack of changes in the amplitude of muscle tone and activity in EMG testing gave rise to the hypothesis that the observed phenomenon could be the result of the desired reorganisation of muscle activity as a result of adaptation to long-term repetitive movement (walking).

6. Analysis regarding the results of haematological blood testing among the examined race-walkers confirmed the correct level of the parameters taken into account; both during the training period and after performing maximal efforts during sports competitions.

7. There were slight fluctuations in haematocrit levels for physiological reasons (commonly known), such as exercise-induced dehydration. This fatigue disorder moved towards the norm at a rate of 30-40% for each day of rest.

8. The unchanging value of the erythrocyte number attracted attention, which correlated with the haematocrit value, further indicating the lack physiological disorders during training and sports competitions.

9. The results of biochemical tests carried out in several series indicated the correct course of adaptation to physical exercise and no grounds for stating non-functional overload of the race-walkers' bodies during training.

10. All the investigated indices concerning blood counts and selected plasma biochemical indicators should therefore constitute a necessary recommendation to conduct periodic examinations in vigorously training race-walkers.

11. The research carried out in this paper may be an indication to define at least 80% of the above-mentioned indicators as model indices, which may guarantee not only the success of athletes, but their health.

12. The proposed model of monitoring nutritional status, consultations, dietary training and the developed individual nutritional plans, combined with supplementation strategies, may serve to optimise training effects and maintain the health of athletes preparing for participation in the above-mentioned top-rank international competitions.

13. Considering the need to provide training race-walkers with an adequate level of antioxidants, mainly vitamins E and C, and reservations about the tendency to exceed (without justification) the recommended standards, they should be advised to focus on implementing a diet rich in fruit, vegetables and other plant-based products rich in antioxidants, which are not found in the manufactured supplements or are selected in single doses.

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Author Contributions
All authors have read and agreed to the published version of the manuscript and agree to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.

Conflicts of Interest
No conflict of interest has to be declared related to this project. No benefits of any kind have or will be received from a third commercial party.

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