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Effect of 2000-meter rowing test on parameters of intestinal integrity in elite rowers during competitive phase - observational study

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Abstract

Background The epithelial wall leakage has been extensively studied in sports disciplines like running and cycling. However, little is known about gut permeability in other disciplines, like rowing, especially after the regular competition performance distance of 2000 m. Therefore, our study aimed to check gut permeability after the 2000-meter rowing test in the annual training cycle. The phenomenon of epithelial wall leakage has been the subject of investigations within athletic domains such as running and cycling. Nevertheless, there exists an insufficiency of understanding regarding gut permeability in alternative disciplines, such as rowing, particularly following the completion of a standard competitive distance of 2000 m. Hence, the principal objective of our study was to assess gut permeability after the completion of a 2000-meter rowing test.

Methods The study was performed at the beginning of a competitive training phase. Eighteen elite rowers of the Polish Rowing Team participated in study after applying the inclusion/exclusion criteria. The participants performed a 2000-meter ergometer test. Blood samples were taken before the test, after exercise, and after 1-hour of restitution. Parameters, such as I-FABP, LPS, LBP, and zonulin, were determined using appropriate biochemical tests.

Results There were no changes between pre- and post-exercise values in I-FABP, LBP, LPS, and zonulin. However, the I-FABP changed from 6,49 ± 2,15 to 8,3 ± 2,71 (ng/ml) during the recovery period, and LBP decreased from 2,73 ± 0,77 to 2,035 ± 0,53 (µg/ml) simultaneously. Other parameters have not changed.

Conclusion The results of this study showed that intense physical effort performed during the training period is sufficient to negatively affect the gut integrity of rowers.

Keywords Athletes, Rowers, Gut permeability, I-FABP, Zonulin, LPS

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Introduction

Exercise–associated symptoms are common among endurance athletes, and they may lead to minor discomfort or cause symptoms of clinical significance [1]. The prevalence of exercise-induced gastrointestinal symptoms among elite endurance athletes was reported to be 70% [2]. Moreover, they are arguably the leading cause of poor performance in competition [3]. Additionally, they may cause of variety of gastrointestinal tract symptoms such as abdominal pain, bloating, the urge to regurgitate, and nausea [4].

The two main pathways may be responsible for exercise-induced gastrointestinal syndrome. The first pathway is connected to the redistribution of blood flow and splanchnic hypoperfusion. In this case, the circulatory-gastrointestinal path diverts blood flow to working muscles and peripheral circulation, reducing total visceral perfusion. The second neuroendocrine-gastrointestinal is connected to stress hormones and results in increased sympathetic activation and concomitant reduction in the functional capacity of the gut. Both pathways may lead to mucosal erosion and damage to the particular cells. In addition, there is a potential third pathway that may strongly influence the gut barrier – mechanical strain: jarring, jolting, position, and friction [1]. Changes in intestinal microbiota caused by intense exercise can lead to gastrointestinal disorders related to exercise [5]. However, recent literature emphasizes that the impact of exercise on gut microbiota depends on factors such as intensity, duration, and chronic adaptation to training. While acute exhaustive exercise can temporarily alter gut microbiota and increase permeability, chronic physical activity is generally associated with positive microbial adaptations, such as increased microbial diversity and enhanced short-chain fatty acid production, which are beneficial for gut health [6]. Karl et al. (2017) demonstrated that multiple-stressor military training-induced increases in intestinal permeability were associated with alterations in inflammation markers and intestinal microbiota composition and metabolism [7]. Moreover, increasing the training loads, duration, and intensity of training may modulate gut microbiota in different ways depending on exercise type, duration, and individual fitness level [8].

As a consequence, gut permeability of the lumen for toxins and bacteria may increase, allowing them to enter the systemic circulation (endotoxemia), burdening the immune system [1, 4]. The translocation of endotoxins may result in endotoxemia and cause sepsis or fatality if left untreated [9]. Moreover, many stressors, such as heat stress, certain medications, and oxidative stress, disrupt gut function and the integrity of the gastrointestinal tract [3]. As a result, gastrointestinal functions, such as motility, digestion, and absorption, may be decreased [1]. The established term "exercise-induced gastrointestinal syndrome" (EIGS) refers to these primary causes and secondary outcomes that naturally arise with the onset and ongoing exercise [1]. Systematic reviews [4, 10] emphasizes that the duration of exercise protocol should last at least 1 h to cause intestinal damage [1, 4, 10]. Researchers' protocols usually focus on two sports disciplines: running and cycling. Still, little is known about other disciplines where exercise protocols are short but on the level of maximum effort. It has been shown that vigorous exercise that lasted not even 10 min has significantly increased intestinal permeability [11]. Gut permeability was also influenced by protocols of running – 20 min at 80% VO2 max (maximal oxygen uptake) [12, 13]. Therefore, a fraction of the anaerobic threshold has been pointed out as a more predictive indicator of gastrointestinal permeability than a fraction of VO2 max, which emphasizes the type of exercise performed does not have as much impact as its intensity [14].

Well-trained rowing competitors cover a distance of 2000 m in about 6 min, reaching the average concentration of lactic acid above 14 mmol/L [15, 16]. Over many years of cooperation with the national team, we have noticed that gastrointestinal disorders occur in 6/24 rowers, which they describe as severe (unpublished data). So far, we have observed increased inflammatory cytokines and reactive oxygen species after the 2000-meter ergometer test [15, 16]. We suspect the above-mentioned changes are accompanied by intestinal damage an increase in their permeability. Additionally, we suspect that the rower's repetitive movements and posture during exercise may strongly influence intestinal damage.

For this reason, we decided to conduct a study that stimulates a rower's race over a typical distance of 2000 m on a rowing ergometer. We hypothesize that although the time of exercise is short, applying such exhaustive exercise will result in gut permeability. As far as we know this is the first study conducted on rowers. Therefore, to determine the presence of intestinal permeability, level of intoxication, and inflammation, markers specific to these changes have been used, such as I-FABP (Intestinal Fatty Acid Binding Protein), zonulin, LPS (Lipopolysaccharide), LBP (lipopolysaccharide-binding protein) to observe both intestinal damage and intoxication. Thus, the study aims to check the influence of the 2000 m maximal test performed on an ergometer on the gut permeability of the rower. The outcomes of the study may be important for trainers and sports dietitians who struggle to improve the performance of the athlete.

The findings of this investigation hold significance for both trainers and sports nutritionists tasked with optimizing athletic performance. Additionally, should brief yet high-intensity exertion impact the integrity of the gastrointestinal barrier, athletes may necessitate heightened

monitoring and support during periods of intensified competition characterized by multiple daily sessions and limited recovery time.

Materials and methods

The study was conducted in July 2022 during an athlete`s camp in a yearly training cycle's competitive phase.

Participants

Eighteen male National Polish Rowing Team members (heavy-weight rowers) participated in this study. Before the exercise test, the anthropometric parameters were assessed using an electronic scale to the nearest 0,05 kg (Tanita BC 418 MA Tanita Corporation, Tokyo, Japan). The study was conducted following the Declaration of Helsinki, and its protocol was approved by the local Ethics Committee at Poznań University of Medical Sciences in accordance with the Declaration of Helsinki (decision no. 314/22 in 2022) and was registered retrospectively under number NCT06372262 registration date 19/03/2024. All participants received an explanation of the study procedures and gave their written, informed consent to participate. Representative study population - the sample size was calculated using a G-power program [17], assuming 0.05 as the alpha level, 0,7 as the effect size (data from our study – not published yet = 0,75, and meta-analysis = 0,81 [10]), and 0.85 power. The minimum sample size was estimated at 13 participants.

Inclusion criteria

Minimum five years of training, five training sessions per week minimum, total training time minimum of 240 min, membership in Polish Rowing Team, finishing 2000-meter ergometer test.

Exclusion criteria

Antibiotic therapy, probiotics, prebiotics within the last three months, dietary regime, and gastrointestinal diseases.

Rowing test

The exercise test was performed at the beginning of a competitive phase of the annual training cycle characterized by high volume and training loads. After baseline blood samples were taken, participants reported to the exercise laboratory after a light breakfast and performed a controlled 2000-meter ergometer test (Concept II, USA). Furthermore, each subject had to finish the distance in the shortest time possible because test results were considered during the selection to the championship team; for that reason, athletes were incredibly motivated to perform test at maximal effort. Before the trial, subjects performed a 5-minute individual warm-up.

Training program during sports camp

The characteristics of a training profile, such as intensity, volume (measured in minutes), and type (specific, i.e., rowing: endurance, technical, speed, etc., and nonspecific: strength, jogging), were recorded daily. The intensity of the training was classified concerning the lactic acid (LA) threshold (4 mmol/L) as an extensive (below the LA threshold) or intensive (above the LA threshold) workload (Table 1). All national team players must comply with the training regime based on the training plan.

Food intake

The total dietary intake was analyzed due to the 24-hour dietary recall method by a dietitian, who was available for participants during all meals. Then, the amount of energy, protein, fat, carbohydrates, and fiber was measured through a commercially available program called DietetykPro (Producer DieteykPro Wrocław, Poland). The above parameters were chosen because they strongly influence the intestinal microbiota, especially carbohydrate, protein and fiber [18]. The subjects used the same canteen where recipes for individual dishes were available, facilitating the quantitative and qualitative consumption assessment. The investigation focused on food consumption within the 24 h preceding the test, acknowledging that the gut microbiome exhibits maximal variability during this temporal window.

Blood sampling and analyses

Blood samples were gained from the antecubital vein before the 2000-meter test (in the morning after an overnight fast), one minute after completion, and after a 1-hour recovery period. The samples were centrifuged after obtaining them to separate serum from blood cells.

Table 1 Training schedule during the week preceding blood sample collection before the test

Days before Trial	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Time rowed, min/day	90	0	70	120	160	80	90	**TEST**
Distance rowed, km/day	20	0	12	22	36	18	20	
Training for force development, min/day	0	0	0	0	0	0	0	
Extensive endurance rowing training time, min/day	64	0	70	120	136	80	90	
Very high intensity endurance rowing training time, min/day	26	0	0	0	24	0	0	
Unspecific training (running, etc.), min/day	90	0	10	30	20	10	10	
Total training time, min/day	**180**	**0**	**80**	**150**	**180**	**90**	**100**	

Table 2 Essential characteristics of study participants ($N = 18$)

	Height [cm]	Weight [kg]	Age [years]	Water [%]	Body fat [%]	Lean body mass [kg]	Training experience [years]
Mean	192,0	89,4	20,3	64,4	9,8	81,0	6,7
SD	4,1	5,8	1,2	2,5	3,0	3,8	1,2

Table 3 Dietary intake

	Energy [Kcal]	Protein[g]	Fat [g]	Carbohydrates [g]	Fiber [g]
Mean	4352,55	181,45	155,56	584,27	33,97
SD	505,29	41,61	34,78	96,31	7,57

The serum was frozen immediately and stored at − 80 °C until testing. In addition, capillary blood samples were obtained from the earlobe before and immediately after the exercise test to assess the athletes' lactic acid levels.

Serum I-FABP, LPS, LBP, and zonulin were measured using commercially available enzyme-linked immunosorbent assays (ELISA; SunRed Biotechnology Company) with an assay range of 0,3–80ng/ml for I-FABP, 12–4000 EU/l for LPS, 0,2–60 µg/ml LBP, and 0,25–70 ng/ml for zonulin. Lactate concentration (La) in capillary blood was checked immediately after sampling using a; lactate concentrations were defined in mmol/L.

Statistical analyses

GraphPad Prism 9 (GraphPad Software, Boston, USA) performed statistical analyses and graphics. Descriptive statistics, such as mean and SD, were used to visualize immediate trends and patterns for three-time points. Shapiro–Wilk test was used to check the normal distribution of variables. The Brown-Forsythe test was used to measure the equality of variances. One-way analysis of variance with repeated measures (ANOVA), with Tukey's posthoc analysis, was used to assess differences in measured variables of the three-time points (pre-exercise, post-exercise, and 1 h recovery), respectively. Zonulin level was not normally distributed, and the Friedman test was used. The t-test was used for La because there were only two-time points. For the effect size measure, Cohen`s d was calculated. Using Cohen`s criteria, the effect size was interpreted as small (0.2), moderate (0.5), and large (0.8) [19]. For correlation analysis, Pearson's coefficient of linear correlation was calculated. The level of significance was for all tests at $p \leq .05$ level. There was no missing data.

Results

Participants

The 2000-meter test on an ergometer is very demanding for a competitor; in addition, 22 rowers have started the test, but only 18 have finished it (one was ill, one refused to row during the race, two couldn't row at this pace) (Table 2).

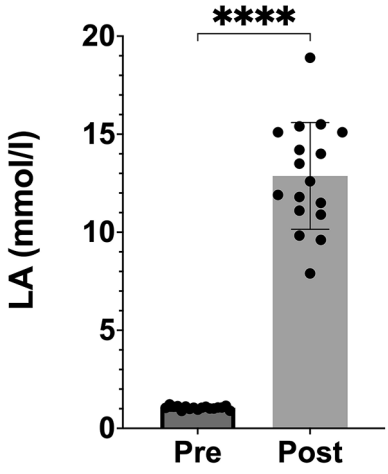

Fig. 1 Effect of 2000-meter test on La levels before and after exercise, values are presented as mean ± SD, significant differences: ****< 0.0001

Dietary intake

The energy intake was 4352 ± 505 kcal, protein intake was approximated at ~ 2,0 g/kg body mass, and fat ~ 1,7 g/kg body mass (Table 3). There was, also negative correlations between carbohydrates and zonulin ($r = -0,763$, $p = 0,006$).

2000-meters rowing test

The average test time was 367,43 ± 7,13, and the mean power output was 453,83 ± 26,45 Wat (5,08 ± 0,08). There was a significant increase in lactate levels observed before and after the exercise test (Fig. 1) ($p < .0001$, Cohen`s d = 8,37). There was no correlation between LA and any other markers.

Markers of gut permeability and inflammation

There were no changes in I-FABP levels after the exercise test and between baseline and recovery (Fig. 2). However, after 1 h, values increased significantly from 6,49 ± 2,15 to 8,3 ± 2,71 (ng/ml) during the recovery period ($p = 0,03$, Cohen`s d = 0,75). There were no changes in LBP level values after exercise and between baseline and recovery, but there was a significant decrease in 1-hour recovery time from 2,73 ± 0,77 to 2,035 ± 0,53 (µg/ml) ($p = 0,045$, Cohen`s d = 1,07). The same pattern occurred at the LPS level, but changes have not reached a significant difference. Zonulin level has not changed in all time points. There were also positive correlations between I-FABP and zonulin ($r = 0,271$, $p = 0,048$) and another between LBP and LPS ($r = 0,331$, $p = 0,014$).

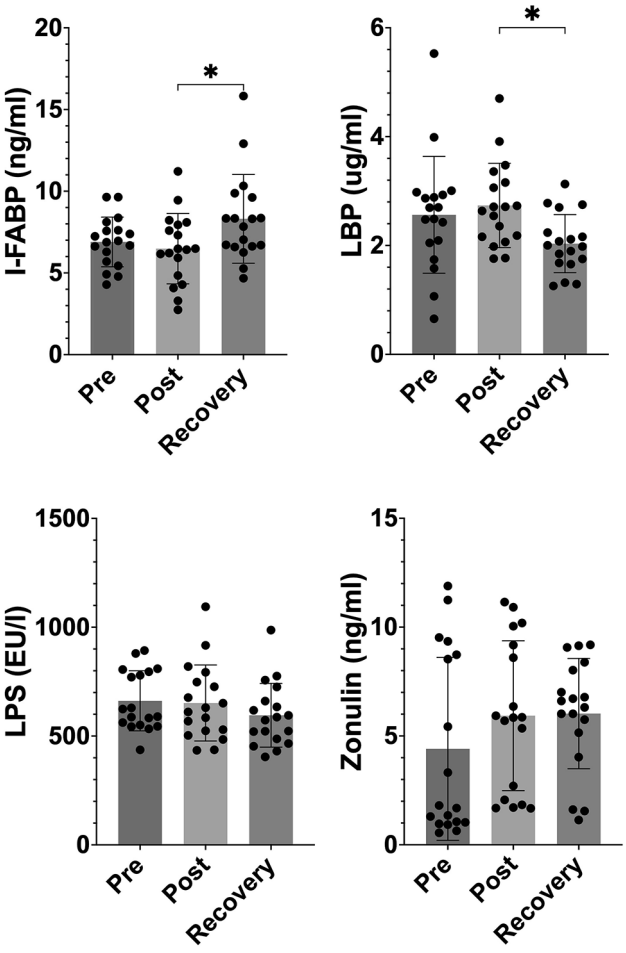

Fig. 2 Effect of 2000-meters test on I-FABP, LBP, LPS, zonulin before, after, and 1-hour after exercise, values are presented as mean ± SD, significant differences *$p < .05$: **< 0.01: ***$p < .001$: ****<0.0001

Discussion

A 2,000-m time trial is a standard test used to assess performance in rowers [20]. We chose the competitive phase because, in this phase, athletes experience strong physiological stresses which is reflected in the increase in biomarkers of tissue damage and immune cell activation [21].

We observed a significant increase in I-FABP levels after the 2000-meter rowing ergometer test. Significant changes concern the time between the post-exercise and recovery period (1 h after), probably due to the low time of exercise (6 min); changes may not have occurred precisely after the end of the ergometer test but are shown 1 h after the recovery. I-FABP is a marker found on the upper luminal surface of the endothelial cell, and its increase reflects endothelial cell injury [10]. It is a sensitive biomarker for early gut damage [22]. Our study's average increase in I-FABP was Δ = 1,81 ng/ml (1810 pg/ml clinical relevance of the I-FABP increase ~ 1000pg/ml) [4]. This response is higher than obtained after 60 min at 70% of the maximum workload capacity [23] or a 20-minute run at a constant speed equivalent to 80% VO_{2peak} [24]. Moreover, running at 78% VO2 max (4 mmol/L blood lactate) until T_c increases by 2.0 °C or volitional exhaustion Δ also gave lower results [25]. Even a prolonged exercise protocol consisting of 15 min cycling at 50% HRR + 60 min running + 15 min cycling at 50% HRR at 30 °C increased I-FABP Δ = 806 pg/ml [26]. Our outcome increase was comparable to 2-hour running at 60% VO2 max Δ = 1230 pg/ml [27]. Though there were no significant changes in zonulin levels after exercise, and the response was strongly individual, there was a positive correlation between I-FABP and zonulin, a marker implicated in regulating mucosal permeability and capable of reversible tight junction disassembly [28]. The correlation observed in our study suggests that as the epithelial injury increased, so did the tight junction leakage. The obtained results may indicate that not only the duration of physical activity but also the intensity is a factor that violates the integrity of the intestinal barrier. Edwards et al. suggest that exercise intensity may be a more decisive factor causing loss of intestinal integrity than exercise mode [14]. Recent reviews emphasise the importance of time as a disturber of the epithelial wall integrity [1, 4, 10]. Still, our results suggest that even a short exercise of very high intensity may increase epithelial injury. These phenomena may give rise to direct consequences, such as alterations to the enteric nervous system and/or enteroendocrine cells, or indirect repercussions, including disruptions to gastrointestinal motility, such as nutrient malabsorption [4]. The main feature of splanchnic hypoperfusion is intestinal ischaemia [23, 29]. In fact, Rehrer et al. reported a decrease in portal blood flow (20%) within the first 10 min of running at 70% of the maximal oxygen update [30]. Furthermore, 1 h of cycling at 70% of maximal wattage out- put (Wmax) resulted in increased splanchnic ischemia, with the most pronounced increase occurring within the first 10 min of exercise [23]. The resultant intense ischemic conditions contribute to epithelial injury, causing erosion across all epithelial cell types and subsequently augmenting intestinal permeability. Remarkably, our study aligns with this temporal pattern, indicating that the initial 10 min of exercise may represent a critical period for the onset of intestinal damage.

Not only intestinal ischaemia but also mechanical strain related to mechanical work performed by the athlete's body during performance may lead to increased I-FABP. For example, runners experience lower GI symptoms (e.g., cramps, bloating) than cyclists [2]. It is found to be a result of the repetitive high-impact mechanics of running [3]. In rowing, we also have repetitive movements that may influence the gut barrier. Posture can also affect gastrointestinal symptoms in rowers. Similar

to cycling, where the cycling position can cause upper gastrointestinal symptoms due to increased pressure on the abdomen. Moreover, the shift of I-FABP could have resulted from changes in the composition of the gut microbiota. Bennett et al. (2020) discovered that a higher relative abundance of both commensal and pathogenic bacterial groups was linked to increased injury of intestinal epithelial cells [31]. Therefore, splanchnic hypoperfusion during exercise, gut microbiota composition, and specific mechanical movements during rowing may have influenced the gut barrier of the rower, and the increase in both parameters suggests increasing gut permeability.

Surprisingly, we observed a decrease in LBP levels after a 1-hour recovery period, without significant changes in LPS levels, but there was a positive correlation between both parameters. Lipopolysaccharide binding protein is mainly synthesized in hepatocytes and epithelial cells [32], and it is considered a stable indirect marker of exposure to bacterial endotoxins [33]. Obtained results in our research are against other authors', where LBP increased after exercise [11, 34, 35] or stayed at the same level [36, 37]. However, an LBP decrease was observed after a 3-hour run [38]. Under conditions of intense physical exertion, LBP may migrate and be transported into cellular compartments [38], which may appear in our study. Moreover, LBP concentration may be connected to the LBP utilization overwhelming the replacement capacity [38]. The reasons for the decreased levels of lipopolysaccharide-binding protein (LBP) after exercise are not entirely understood. Motiani et al. (2019) found that after two weeks of training, there was a significant reduction in LBP levels, along with a modification in the microbiota profile, characterized by an increase in the Bacteroidetes phylum. At the species level, Bacteroidetes showed a negative correlation with plasma inflammatory markers, including LBP, TNF-α, and CRP levels [39]. This substantial decrease in LBP may be attributed to changes in the gut microbiota. As observed in our study, LBP decrease was probably associated with the physical exertion of rowers during the 2000-meter ergometer test and connected to both migration into cellular compartments and overwhelmed replacement capacity or shift in gut microbiota composition.

Remarkably, our observations revealed a negative correlation between zonulin levels and carbohydrate intake. This finding aligns with the results reported by Etxebarria et al., where carbohydrate intake 24 h before exercise demonstrated an association with reduced lipopolysaccharide translocation [37]. Notably, a judicious carbohydrate intake in the 24 h preceding exercise may contribute to mitigated gut permeability. These findings underscore the potential impact of dietary choices on modulating biomarkers associated with gut health, emphasizing the significance of pre-exercise nutritional strategies in influencing gastrointestinal outcomes.

An intensive 2000-meter ergometer test simulates the start of a rower in a competition. This is the only starting distance for the male rowers. Hence, sustained elevation in gut permeability markers, persisting even after a one-hour recovery period, could potentially render rowers susceptible to gastrointestinal symptoms. This, in turn, may lead to decrease performance outcomes at the start, necessitating prolonged recovery periods, and in severe instances, disqualification from competition. Consequently, implementing effective nutritional strategies and supplementation regimens becomes pivotal in preserving athletes' optimal physical condition.

Practical applications

The findings of this study are highly relevant for trainers and sports nutritionists aiming to enhance athletic performance. If short bursts of high-intensity exercise compromise the gastrointestinal barrier, athletes may require increased monitoring and support during demanding competition phases with multiple daily sessions and minimal recovery. Practical strategies, such as adjusting pre-exercise nutrition or implementing microbiota-targeted interventions, could be crucial in addressing these challenges.

Conclusion

The primary discovery of the study reveals that even a brief duration of maximal exertion, such as 6 min, can impact the integrity of the gut barrier. Notably, the intensive 2000-meter ergometer test undertaken by highly proficient rowers elicited alterations in observed parameters. Elevated levels of intestinal fatty acid-binding protein (I-FABP), concurrent with heightened zonulin levels, imply a compromised gut barrier integrity attributed to both enterocyte damage and disruption of tight junctions. Furthermore, the effect of training loads and the administered test appears to contribute to the observed decline in lipopolysaccharide-binding protein (LBP) levels, accompanied by a reduction in lipopolysaccharide (LPS) levels during the recovery phase. This phenomenon may be attributed to the sequestration of LBP into cellular compartments and an insufficient capacity for replacement. The study's outcomes underscore that intense physical exertion during training regimens is adequate to impact rowers' gut permeability negatively. Such insights are invaluable for coaches, sports nutritionists, and medical professionals striving to enhance the exercise capacity of rowing athletes.

Study limitations

The present study acknowledges certain limitations, foremost among them being the relatively small sample size. Conducting research within the domain of elite sports poses challenges in assembling a cohort of highly trained athletes with comparable performance levels and training regimens. Furthermore, a more comprehensive

exploration of inflammatory markers in subsequent studies is imperative to gain a thorough understanding of the observed changes. Another limitation stems from the absence of individualized breakfast analyses; however, it is noteworthy that the breakfast was standardized to meet energy and macronutrient requirements. Future investigations would benefit from incorporating detailed information on standardized meals into their protocols for a more nuanced analysis of nutritional influences.

Abbreviations

EIGS	Exercise-induced gastrointestinal syndrome
ELISA	Enzyme-linked immunosorbent assays
HRR	Heart rate recovery
HSP	Heat shock proteins
I-FABP	Intestinal fatty acid binding protein
La	Lactic acid
LBP	Lipopolysaccharide-binding protein
LPS	Lipopolysaccharide
MNC	Mononuclear cells
ROUT	Method combines robust regression and outlier removal
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TLR	Toll-like receptors
TNF-alfa	Tumor necrosis factor alfa
VO2max	Maximal oxygen uptake

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Author contributions
"H.D. and ASS conceived and planned the experiment. A.K., JCW, and JOK carried out laboratory analysis. H.D. contributed to the statistical analysis and took the lead in writing the manuscript with consultation with ASS, HD, PB collected the data, HD visualization, ASS and HD project administration. All authors have read and agreed to the published version of the manuscript."

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Data availability
"Due to ethical concerns, the datasets generated and/or analyzed during the current study supporting data cannot be made openly available. However, they are available from the corresponding author upon reasonable request."

Declarations

Ethics approval and consent to participate
The study was conducted following the Declaration of Helsinki, and its protocol was approved by the local Ethics Committee at Poznań University of Medical Sciences (decision no. 314/22 in 2022). All procedures and potential risks were discussed with the participants before the study. Furthermore, informed consent was obtained from all parents or legal guardians and subjects before participation in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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