Original paper

The impact of a one-time workout at a temperature of –10°C on serum proteins in young men

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Key words: single effort, beep test, air temperature –10°C, thermal climate chamber, protein profile.

Abstract

Introduction: A single physical exertion rapidly evokes cardiovascular changes. Owing to the body's ability to maintain homeostasis, it is able to respond to increased metabolic demand.
Aim of the research: The aim of this study was to assess the effect of a single physical exercise performed at low temperatures (–10°C) on changes in blood serum protein profile, and to compare the results with a control group that performed the same activity in atmospheric conditions of 18°C.
Material and methods: The participants of the study were divided into two groups: Group 1 (study group, n = 15) ran in a thermoclimatic chamber at an air temperature of –10°C, and Group 2 (control, n = 15) ran outdoors at an air temperature of 18°C. In group 1, the subjects (n = 15) performed a 5-minute warm-up in standing position, which was carried out before entering the thermoclimatic chamber of the Laboratory of Technoclimatic and Working Machines (LBT&MR) of the Faculty of Mechanical Engineering at Krakow University of Technology (Poland), at an air temperature of –10°C, and then participated in a beep test endurance shuttle run conducted until exhaustion under the supervision of a trainer. In Group 2 (n = 15), the subjects performed the same test protocol, but outdoors, with an air temperature of 18°C.
Results: Blood samples were collected from the subjects before entering the thermal climate chamber and after leaving it (fasting, from the antecubital vein, volume 5 ml, into tubes) by a qualified nurse. The blood samples were analysed in the Diagnostyka S.A. laboratory in Krakow, Poland for protein electrophoresis.
Conclusions: Prevailing temperature conditions during physical exercise had no substantial influence on the changes in analysed the blood parameters. An exception was β1-globulin [%], in this instance, a substantial impact of the experimental factor (air temperature –10°C) was shown, indicating that the increase in β1-globulin in low temperatures is primarily relative, associated with haemoconcentration rather than an actual increase in protein synthesis.

Introduction

It has been suggested that a single bout of physical exercise at low temperatures may cause changes to the cardiovascular system [1–3]. It is becoming increasingly common to use cold acclimatisation to improve athletic performance, especially in endurance and high-intensity training. Scientific studies have shown that moderate exposure to cold may induce several physiological adaptations that can improve athletic performance [4]. It is believed that the human organism's ability to maintain homeostasis enables it to respond to an increased metabolic demand [5–7].

Proteins are complex macromolecules that play a vital role in numerous key functions in the human organism. These elements perform regulatory, transport, catalytic, and immunological functions. They are the building elements of body cells and form the cytoskeleton, which is responsible for maintaining shape and connectivity within a cell. Proteins dissolved in plasma perform several vital functions in the human body. One of the primary functions of albumin is to facilitate the transportation of fatty acids and steroid hormones between body tissues.

The available literature does not provide data on the impact of running at low temperatures on changes in the serum protein profile. Changes in serum protein levels have only been demonstrated in other sports disciplines. For example, Markiewicz and Kubasiewicz-Ujma (1977) assessed 156 men aged 23–24 years for the impact of progressively increasing physical exertion on a bicycle ergometer [8]. They found changes in all protein fractions caused exclusively by blood thickening. Duda *et al.* (2003) obtained similar results in their study of changes in serum protein concentrations under the influence of intense physical exercise in young male blood donors [9]. The studies were conducted on two occasions: before and 24 hours after the donation of 450 ml of blood. External conditions (temperature, humidity, time of day) were meticulously maintained during both study sessions, conducted 7–12 days apart. Thirteen participants underwent rigorous physical exercise on a bicycle ergometer until reaching the point of exhaustion. Blood samples were collected on three occasions: immediately before and after the exercise, and 2 hours after the end of the activity. Duda *et al.* (2003) demonstrated in their research that intense physical exercise caused a 6–8% increase in total protein concentration, while the increase in the concentrations of other serum protein fractions was 5–15% [9]. These changes were observed both before and after the participants donated 450 ml of blood. Prior to the donation, two hours after the exercise test, the concentrations of all serum proteins returned to normal levels. When the exercise test on a bicycle cycloergometer was performed after a donation of 450 ml of blood, levels of total protein, α1-globulin, and γ-globulin fractions 2 hours after the exercise were statistically significantly lower compared with baseline values. In turn, Teległów *et al.* (2022), in their analysis of changes in immunoglobulin levels (IgG, IgA, IgM) and protein electrophoresis, observed a downward trend for total protein, albumin, globulin, IgG, IgA, and IgM levels [10]. This may reflect an inhibitory effect of intense physical exercise on the immune system. The decrease in the values of all protein fractions caused by extreme physical exercise, such as triathlon, is probably due to an increase in stress hormone levels (adrenaline and cortisol). These hormones have an inhibitory effect on the immune system. Regular physical activity, which is a prerequisite for adapting the body to the stresses associated with triathlon competitions, improves metabolism. Despite the demonstrated changes in average serum protein concentrations after triathlon competitions, Teległów *et al.* (2022) observed that all protein levels were within the reference ranges for the general population [10]. This suggests efficient adaptive mechanisms of the body and testifies to the good health of the triathletes studied.

Aim of the research

The objective of this study was to evaluate the impact of a single physical exercise session conducted in low-temperature conditions (–10°C) on changes in the blood serum protein profile, and to compare the results with a control group that performed the same activity in standard atmospheric conditions at a temperature of 18°C.

Material and methods

Characteristics of the study population

The study involved 30 young men (aged 22 years) who were third-year physiotherapy students at the University of Physical Culture in Krakow, Poland, in the 2023/2024 academic year. The inclusion criteria for the study were as follows: subjects were required to be aged 22 years, male, not to have undergone competitive training in any sport, and free of chronic diseases or contraindications to physical activity. Participants were excluded from the study if they met any of the following criteria: age above or below 22 years, chronic cardiovascular or respiratory diseases, eating disorders, symptoms of infection (fever, cough, rhinitis), or competitive participation in any sport. The participants were also informed in detail about the purpose and procedure of the study, as well as the fact that they could withdraw from participation at any time without any consequences.

Study groups

The young men who participated in the study were divided into two groups:
– Group 1 (the study group [n = 15]) underwent a running session within a thermal chamber that was maintained at an air temperature of –10°C.
– Group 2 (the control group [n = 15]) engaged in running activities outdoors in an environment at air temperature of 18°C.

Intervention

In Group 1, the subjects (n = 15) performed a 5-minute warm-up in a standing position. This was carried out prior to entering the thermoclimatic chamber of the Laboratory of Technoclimatic and Working Machines (LBT&MR) of the Faculty of Mechanical Engineering at Krakow University of Technology, at an air temperature of –10°C. The subjects then took part in an endurance shuttle run conducted until exhaustion under the supervision of a trainer (Figure 1).

Effective training consists of exercise at an intensity of 80% for a duration of at least 10 minutes, Kozłowski *et al.* (1999). The thermoclimatic chamber had accreditation certificate No. AB 1678, issued by the Polish Centre for Accreditation, and fulfilled the requirements of the PN – EN ISO/IEC 17025:2005 standard. The Technoclimatic and Working Machinery Research Laboratory is a research facility accredited in the field of defence and security (O&B) by the Minister of National Defence (accreditation No. 55/MON/2018).

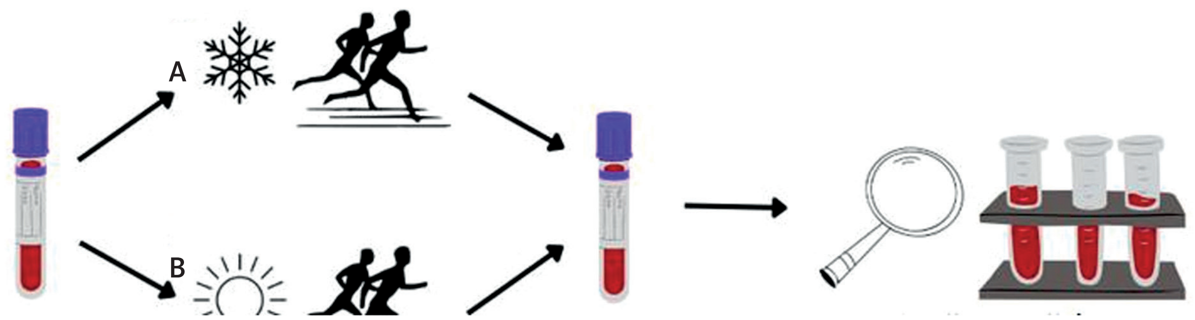

Figure 1. Course of the study: **A** – study group, **B** – control group

In Group 2, the subjects (n = 15) performed a 5-minute warm-up in a standing position, which was carried out in natural environmental conditions at an air temperature of 18°C. They then took part in a beep test conducted until exhaustion under the supervision of a trainer.

The methodology for conducting the endurance shuttle run test with progressively increasing intensity (20 m shuttle run test [20MST]) was consistent for both the study and control groups. The beep test, a tool for the assessment of cardiorespiratory fitness, commenced with a run at a relaxed pace of 8 km/h. The speed attained by the subjects at the conclusion of the experiment was contingent on individual factors, including the number of stages completed. The participants traversed 20-metre segments in accordance with the pace dictated by an auditory signal – a whistle. The completion of a section was determined by the subject's contact with the end line with a single foot. The sound signal was subject to a frequency modulation of 0.5 kHz per minute, which resulted in an increase in running speed by 0.5 km/h. The test was interrupted when a participant was unable to cross the line and was at least 3 m away from it (despite the sound signal) for two consecutive sections. The result of the test was the number of successfully completed sections [11].

Research method

Blood samples were collected from the subjects prior to their entry into the thermal climate chamber and following their exit from it (fasting, from the antecubital vein, volume 5 ml, into tubes) by a qualified nurse. The blood samples were analysed in the Diagnostyka S.A. laboratory in Krakow, Poland. Protein electrophoresis was performed in a Cobas c 311/511 analyser from Roche/Hitachi systems at the Diagnostyka S.A. laboratory in Krakow, Poland. The study was approved by the Ethics Committee of the Regional Medical Chamber in Krakow, Poland (approval No. 171/KBL/OIL/2023) and was conducted in accordance with the principles set out in the Declaration of Helsinki.

Statistical analysis

The data analysis was conducted using Statistica 13.1 software (StatSoft Polska). The differences between the measurements taken before and after the intervention, as well as between the study and control groups, were analysed using two-factor analysis of variance with repeated measurements (ANOVA). Tukey's Honestly Significant Difference (HSD) test was used for multiple post-hoc comparisons, with additional verification using Bonferroni correction. The normality of the distribution was assessed using the Shapiro-Wilk test. The homogeneity of variance was checked using Levene's test. Mauchly's test was used to evaluate the sphericity of variance. The effect size was calculated using Cohen's d coefficient. The effect size of the analysis of variance was assessed using partial eta squared. A probability of type I error below 0.05 ($p < 0.05$) was assumed as the level of statistical significance.

Results

A single exercise session at a low temperature of –10°C, conducted in the form of an endurance shuttle run with progressively increasing intensity (beep test), was shown to have no effect on changes in serum protein levels when compared to the control group, which performed the same running test in a natural environment at temperature of 18°C (Table 1). The study results indicate that the observed changes remained within reference ranges and were primarily of physiological rather than clinical significance.

Table 2 presents the results of the normality distribution tests for variables related to physical exercise. The analysis showed that the distribution of values for duration of exercise and estimated maximal oxygen uptake (VO_2max) in both study groups did not deviate from normality.

Statistically significant changes were demonstrated for increase in total serum protein levels ($p < 0.001$ in both groups), increase in albumin levels in the study group ($p = 0.006$) and in the control group ($p = 0.003$),

Table 1. Determination of the level of statistical significance for differences between the study group and the control group after the running test

Comparison of statistical significance in the study and control groups		*P* for study group after test – control group after test
Total protein	[g/l]	0.978
Albumin	[%]	0.996
α1-globulin	[%]	0.931
α2-globulin	[%]	0.901
β1-globulin	[%]	0.937
β2-globulin	[%]	0.677
γ-globulin	[%]	0.993
Albumin	[g/l]	> 0.999
α1-globulin	[g/l]	0.979
α2-globulin	[g/l]	0.973
β1-globulin	[g/l]	> 0.999
β2-globulin	[g/l]	0.612
γ-globulin	[g/l]	0.981
Albumin/globulin	[n]	0.973

**All p-values > 0.05.*

increase in β1-globulin levels in the study group (p = 0.002) and in the control group (p = 0.040), and increase in β2-globulin levels in the study group (p < 0.001) and in the control group (p = 0.001) (Tables 3, 4).

Furthermore, statistically significant changes were observed in the study group, including increases in α1-globulin and α2-globulin levels (p = 0.006), an increase in γ-globulin levels (p = 0.001), and a decrease in the albumin/globulin ratio (p = 0.015) (Tables 3, 4).

Figure 2 presents the partial eta-squared values for the analysed protein levels. This coefficient was used to ascertain the effect size in the analysis of variance. It demonstrates the relationship between the dependent variable and the predictors. It is a measure of the extent to which the independent variables explain the dependent variable. In most protein levels, a substantial and statistically significant effect of the measurement factor was identified. The running exercise test was found to have a significant effect on the subjects from both groups. Regarding the group factor, no statistically significant values of the partial eta-squared coefficient were identified. The temperature conditions during physical exertion had no significant effect on the observed changes in blood protein levels, with the exception of β1-globulin [%], for which a large, statistically significant effect was identified at a level of p = 0.040 for the interaction between the measurement factor and the group. In this case, a significant effect of the experimental factor of an air temperature of –10°C was demonstrated.

Discussion

The aim of this study was to evaluate the impact of a beep test exercise performed in low-temperature conditions (set at –10°C) on alterations to the blood serum protein profile, and to compare the results with a control group that performed the same activity in atmospheric conditions of 18°C. This study should be regarded as a pilot study, providing a solid foundation for future, more comprehensive research projects. A single exercise at a low temperature of –10°C in the form of an endurance shuttle run with progressively increasing intensity (beep test), in comparison to a control group that performed the same running test in a natural environment at a temperature of 18°C, indicated that the observed changes remained within reference ranges and were primarily of physiological rather than clinical significance. The present study is characterised by limited statistical power due to the small sample size (n = 15). Consequently, the lack of significant differences between the groups may result from a sample size error rather than the absence of a relationship between the variables. The findings indicate that the prevailing temperature conditions during physical exertion had no significant effect on the changes of the assessed blood parameters. An exception was β1-globulin, for which a large, statistically significant effect was found at p = 0.040 for the interaction between the measurement factor and the group. In this case, a significant effect of the experimental factor (air temperature –10°C) was demonstrated, suggesting that the increase in β1-globulin during cold exposure is primarily relative, due to haemoconcentration rather than an actual increase in protein synthesis.

Minor fluctuations in serum protein concentrations during periods of chronic exercise can be attributed to the peri-exercise dehydration that predominantly affects interstitial fluid and intracellular fluid, with a minor effect on plasma [12]. Other authors argue that an increase in the g-globulin fraction, concomitant with blood thickening, is precipitated

Table 2. Assessment of normality for duration and VO_2max variables using the Shapiro-Wilk test

Parameter	Study group		Control group	
	W [unit]	*P*-value	W	*P*-value
Duration	0.946	0.463	0.909	0.152
VO_2max	0.943	0.419	0.939	0.412

Table 3. Blood protein levels before and after the running test in the study group

Study group		**Mean ± SD before test**	**Mean ± SD after test**	***P* before – after**
Total protein	[g/l]	73.14 ±2.42	80.00 ±2.57	**< 0.001***
Albumin	[%]	62.51 ±4.09	59.84 ±5.28	**0.028 ***
α1-globulin	[%]	2.11 ±0.24	2.23 ±0.40	0.189
α2-globulin	[%]	9.91 ±0.98	10.51 ±1.39	0.080
β1-globulin	[%]	7.79 ±1.12	8.40 ±1.24	**0.002***
β2-globulin	[%]	4.71 ±1.12	5.15 ±1.54	0.165
γ-globulin	[%]	13.01 ±2.74	13.89 ±2.68	0.085
Albumin	[g/l]	45.71 ±3.02	47.85 ±4.06	**0.006***
α1-globulin	[g/l]	1.55 ±0.20	1.78 ±0.33	**0.006***
α2-globulin	[g/l]	7.25 ±0.81	8.43 ±1.21	**< 0.001***
β1-globulin	[g/l]	5.67 ±0.80	6.70 ±1.01	**< 0.001***
β2-globulin	[g/l]	3.43 ±0.77	4.13 ±1.28	**0.001***
γ-globulin	[g/l]	9.54 ±2.17	11.13 ±2.25	**0.001***
Albumin/globulin	[n]	1.70 ±0.28	1.52 ±0.29	**0.015***

**Statistical significance (p ≤ 0.05).*

Table 4. Blood protein levels before and after the running test in the control group

Control group		**Mean ± SD before test**	**Mean ± SD after test**	***P* before – after**
Total protein	[g/l]	75.51 ±4.04	80.24 ±4.51	**< 0.001***
Albumin	[%]	60.46 ±2.43	60.01 ±3.09	0.943
α1-globulin	[%]	2.10 ±0.36	2.16 ±0.35	0.900
α2-globulin	[%]	10.10 ±0.62	10.10 ±0.72	> 0.999
β1-globulin	[%]	8.07 ±0.59	8.14 ±0.77	0.740
β2-globulin	[%]	8.07 ±0.59	5.43 ±0.93	0.541
γ-globulin	[%]	13.87 ±2.54	14.08 ±2.46	0.985
Albumin	[g/l]	45.3 ±2.12	48.06 ±2.03	**0.003***
α1-globulin	[g/l]	1.59 ±0.30	1.74 ±0.29	0.193
α2-globulin	[g/l]	7.63 ±0.60	8.11 ±0.81	0.056
β1-globulin	[g/l]	6.19 ±0.56	6.60 ±0.85	**0.040***
β2-globulin	[g/l]	3.98 ±0.75	4.37 ±0.87	**0.018***
γ-globulin	[g/l]	10.51 ±2.21	11.36 ±2.43	0.078
Albumin/globulin	[n]	1.54 ±0.16	1.52 ±0.19	0.968

**Statistical significance (p ≤ 0.05).*

by increased synthesis of γ-globulin by plasma cells, the reticuloendothelial system, and lymphoid tissue (Markiewicz *et al.* 1977). In contrast, other studies have found that, despite plasma thickening, there were no changes in albumin concentration. This is probably related to the process of increased albumin excretion in urine during physical exercise and increased albumin catabolism ((Markiewicz *et al.* 1977). According to Nelsen *et al.* (2023), acute high-intensity interval exercise increased plasma volume 24 hours post-exercise [13]. An upright posture during physical exertion plays a role in increasing plasma volume due to the impact on lymphatic drainage and albumin redistribution, while exercise in a recumbent position has no such effect. The present study indicates that a single exercise session in the form of a beep test endurance shuttle run with progressively increasing intensity leads to an increase in serum protein concentrations, which occurs in the case of plasma thickening. The reduction in blood plasma volume during exercise is the re-

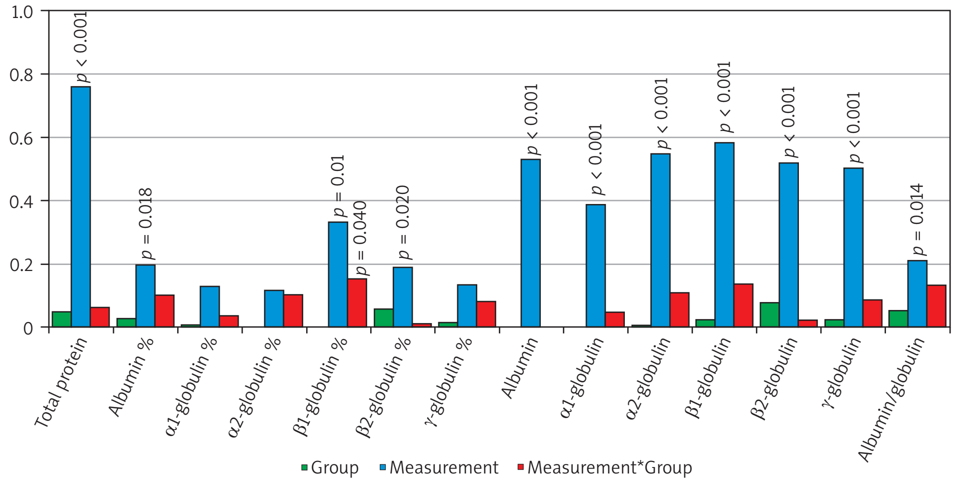

Figure 2. Partial eta-squared values for the analysed protein levels

sult of fluid shifts between the extracellular and intracellular compartments due to thermoregulation. This regulatory mechanism entails the transfer of water from plasma to the exercise-stressed skeletal muscle cells. The beep test has been demonstrated to result in an increase in protein fraction concentrations in both the study and control groups, which in both low- and high-temperature conditions is primarily relative and results from haemoconcentration. In low temperatures, the dominant mechanisms are fluid redistribution and peripheral vasoconstriction, whereas in high temperatures, the main cause is sweat-induced water loss and dehydration. Changes induced by a single bout of physical effort – the beep test performed to exhaustion in a thermoclimatic cryochamber at –10°C – indicate an increase in the concentrations of total protein, albumin, β1-globulin, and β2-globulin, as typically seen in cases of haemoconcentration. Similar changes were observed in the group performing the beep test to exhaustion outdoors under ambient conditions (18°C). These variations are metabolic and circulatory in nature and result primarily from increased oxygen and energy demands.

However, the findings revealed that the temperature conditions during physical exertion had no significant influence on the changes in blood protein levels. The exception to this was β1-globulin [%], for which a large, statistically significant effect was identified at $p = 0.040$ for the interaction between the measurement factor and the group, indicating that the increase in β1-globulin in low temperatures is primarily relative, associated with haemoconcentration rather than an actual increase in protein synthesis. In this instance, a substantial impact of the experimental factor (air temperature –10°C) was shown. A study by Pavlinovic *et al.* (2024) assessed the effect of ambient temperature on the running performance of football players participating in the UEFA Champions League [14]. The authors observed that at an air temperature ranging from 6 to 10°C, soccer players covered greater distances during matches and undertook more high-intensity and high-speed runs (including sprints) compared to matches played at temperatures ≥ 21°C. However, this study did not assess the impact of physical exertion under varying temperature conditions on changes in the blood protein profile.

Similar research was conducted by Morgans *et al.* (2023), who assessed the impact of environmental conditions on key physical abilities of soccer players [15]. As they noted in their work, the extant literature does not provide data on the impact of negative air temperatures on the athletic performance of elite soccer players. Following a comprehensive analysis of 1142 matches played between 2016 and 2021, they demonstrated that the total running distance achieved during a match at an ambient temperature of up to 10°C was statistically significantly higher than at temperatures from 11–20°C and > 20°C. However, Morgans *et al.* (2023) observed a statistically significant reduction in sprinting distance at temperatures below –5°C compared to the same activity performed at higher temperature ranges [15].

In summary, further investigation is required to assess the effect of physical exercise in the form of endurance shuttle running with progressively increasing intensity on individual protein fraction levels. Such research would establish and verify the impact of low air temperature on the body.

Conclusions

The study represents a pilot contribution to the field of exercise physiology under low-temperature conditions and should be regarded as a solid foundation for future, more comprehensive research projects.

The present study is characterised by limited statistical power due to the small sample size ($n = 15$). Consequently, the lack of significant differences between the groups may result from a sample size error rather than the actual absence of a relationship between the variables.

Changes induced by a single bout of physical effort – the beep test to exhaustion performed in a thermoclimatic cryochamber at –10°C – indicate an increase in the concentrations of total protein, albumin, β1-globulin, and β2-globulin, as typically seen in cases of haemoconcentration. Similar changes were observed in the group performing the beep test to exhaustion outdoors under ambient conditions (18°C). These variations are metabolic and circulatory in nature and result primarily from increased oxygen and energy demands. The study indicates that the observed changes remained within reference ranges and were primarily of physiological rather than clinical significance.

The findings indicate that the prevailing temperature conditions during physical exercise had no substantial influence on the changes in analysed the blood parameters. An exception was β1-globulin [%], for which a substantial statistically significant effect was identified at $p = 0.040$ for the interaction between the measurement factor and the group. In this instance, a substantial impact of the experimental factor (air temperature –10°C) was shown, indicating that the increase in β1-globulin in low temperatures is primarily relative, associated with haemoconcentration rather than an actual increase in protein synthesis.

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Ethical approval

The study was approved by the Ethics Committee of the Regional Medical Chamber in Krakow, Poland (approval No. 171/KBL/OIL/2023) and was conducted in accordance with the principles set out in the Declaration of Helsinki.

Conflict of interest

The authors declare no conflicts of interest. The funder played no part in the study’s design, data collection, analysis, interpretation, or manuscript writing. The decision to publish the results was also not influenced by the funder.

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