

Original Article

Relationship Between Blood Creatine Kinase and Muscle Damage in Speed Skaters

Takanori Matsuura¹, Masaki Yamaguchi², Koichiro Kuwahara³, Koji Yoshie³, Yukari Okuma³, Masahiro Yuki¹

¹Graduate School of Medicine, Science and Technology, Shinshu University, Nagano, Japan;

²Graduate School of Medicine, Science and Technology, Shinshu University, Ueda, Japan;

³Department of Cardiovascular Medicine, Shinshu University School of Medicine, Matsumoto, Japan

Abstract

Objective: This study aimed to explore whether resting creatine kinase (CK) levels are associated with a combination of commonly used psychophysiological parameters in speed skaters. Speed skaters were regarded as ideal candidates for this study, considering the sport's high incidence of muscle damage. **Methods:** Participants consisted of nine speed skaters, including international-level competitors, and nine non-athletes who served as controls. Measurements included CK, ferritin (Fer), perceived fatigue level (PFL), sleep quality assessment (SQA), international physical activity questionnaire (IPAQ), pulse rate, blood pressure, physical activity, thigh circumference, handgrip strength, and jump height. A multiple regression analysis was performed with CK as the dependent variable and the other psychophysiological parameters as independent variables. **Results:** CK, IPAQ, thigh circumference, and jump height were significantly higher in speed skaters ($p < 0.05$), whereas pulse rate and PFL were significantly lower ($p < 0.05$). In an exploratory multiple regression analysis, a combination of pulse rate, mean arterial pressure, PFL, and SQA showed the highest coefficient of determination ($R^2 = 0.54$), suggesting that these variables may be associated with inter-individual variation in resting CK levels. **Conclusion:** The combination of conventional psychophysiological parameters was associated with variation in CK levels. These findings provide preliminary evidence that CK may be a plausible biomarker of muscle damage in elite speed skaters.

Keywords: Athlete, Biomarker, Muscle Damage, Speed Skater

Introduction

Exercise plays an essential role in the healthy physical and mental development of children and adolescents. However, sports-related injuries represent a major public health concern worldwide. In Japan, approximately 266,000 cases occur per year among elementary school students, 231,000 per year among junior high school students, and 183,000 per year among high school students in a population of 120 million¹. The social and

economic loss resulting from these injuries is considerable. Despite this situation, there remains insufficient evidence regarding the optimal training volume and methods that can enhance athletic performance without causing injuries. Consequently, it has been reported that training practices have often relied heavily on the experience and intuition of coaches².

Recent advances in information technology have enabled continuous monitoring of athletes' performance using accelerometer-based motion capture³ and heart rate measurements⁴, allowing real-time analysis of training responses. These approaches have been applied to provide immediate feedback on training effects, prevent overtraining, and optimize nutritional management. In addition, biochemical measurement of blood markers has demonstrated high diagnostic utility, and the use of sports injury biomarkers has recently been proposed as a promising approach for preventing musculoskeletal injuries^{5,6}. CK, which reflects microdamage in skeletal muscle, has been

The authors have no conflict of interest.

Corresponding author: Masahiro Yuki, Shinshu University, Faculty of Education, 6-ro Nishinagano, Nagano 380-8544, Japan
E-mail: myuki@shinshu-u.ac.jp

Edited by: G. Lyritis

Accepted 9 January 2026



Table 1. Subject characteristics and competitive levels.

Subject	Age	Sex	Height	Body weight
S-1	19	M	1.70	68.2
S-2	20	F	1.72	72.1
S-3	20	M	1.64	64.6
S-4	21	F	1.60	69.4
S-5	23	M	1.74	72.4
S-6	24	M	1.51	57.1
S-7	26	M	1.76	75.0
S-8	27	F	1.56	53.1
S-9	34	M	1.77	77.2
C-1	29	M	1.70	72.8
C-2	21	F	1.68	58.9
C-3	20	M	1.63	58.7
C-4	22	F	1.63	64.4
C-5	22	F	1.81	70.0
C-6	23	M	1.48	38.6
C-7	22	M	1.70	65.5
C-8	26	F	1.63	49.1
C-9	34	M	1.83	78.9
Speed skaters	24.0 ± 4.3		1.66 ± 0.08	67.7 ± 7.6
Controls	24.3 ± 4.3		1.67 ± 0.10	61.9 ± 11.6
<i>p</i> value	0.893		1.000	0.387

S: Speed skaters, C: Controls.

widely used in the literature as a biomarker related to muscle recovery; when CK levels return to baseline promptly each day, it may prevent the accumulation of fatigue and the onset of muscle damage⁷. Serum ferritin, Fer, a key indicator of anemia, has traditionally been considered unrelated to sports injuries. Iron deficiency, however, can reduce collagen strength, and maintaining adequate iron stores may help prevent sprains and ligament or tendon injuries⁸. Monitoring these biomarkers has been trialed in managing elite athletes, including participants in the World Cup and Olympic Games^{9,10}.

Despite their considerable potential impact on the prevention, diagnosis, and treatment of sports injuries, biomarker-based assessments have not yet become widely available for routine use by athletes themselves. A major reason is the wide variability in muscular load characteristics across different sports. The primary muscles involved, as well as the magnitude and duration of mechanical load, vary greatly depending on the sport discipline, the athlete's age, sex, level of skill, muscle mass, and ethnic background. We have been focusing on improving performance in sprint speed skating, which requires extremely high explosive power. Elevated CK levels are observed even after skating sessions¹¹, and previous studies have reported that the risk of injury increases 2.56 times when CK levels exceed

467 U/L in speed skaters¹². Although several studies have examined individual biomarkers such as CK or Fer in speed skaters, few have adopted an integrated approach that simultaneously considers subjective indices, physiological parameters, morphological characteristics, and physical performance measures to explore an integrated framework to describe the athlete's psychophysiological state.

The purpose of this study was to explore whether resting CK levels are associated with a combination of multiple psychophysiological parameters commonly used in athlete evaluation in elite speed skaters. Accordingly, we selected speed skaters as participants because the event they performed was characterized by short-duration, high-intensity performance requiring explosive power, which is associated with a high frequency of muscular microinjuries and a notable influence of anemia. To ensure the quality of the dataset for hypothesis testing, top-level Japanese athletes, including international competitors, were recruited. Data were collected from multiple perspectives, including biochemical markers, CK and Fer, subjective indices such as PFL and SQA, and physical activity level assessed by the IPAQ-short version, IPAQ. Physiological parameters such as pulse rate and blood pressure, and physical performance measures including physical activity, thigh circumference, handgrip strength, and jump height,

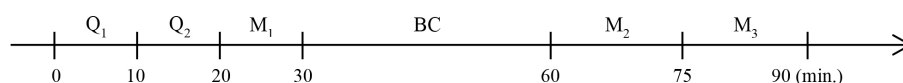


Figure 1. Experimental protocol for biomarker and participant characteristic analysis (Q1: Questionnaire administration regarding exercise habits, sleep quality, and perceived fatigue levels, Q2: IPAQ-SV administration, BC: Blood sample collection, M1: Measurement of seated blood pressure (systolic/diastolic) and pulse rate, M2: Measurement of thigh circumference, M3: Measurement of grip strength and Counter Movement Jump (CMJ) height).

were also measured. Multiple regression analysis was performed to explore associations between CK levels and multiple psychophysiological parameters.

Methods

Participants

Eighteen participants took part in this study, comprising nine speed skaters (five male and four female, aged 19 – 35 years) and nine non-athletic controls (five male and four female, Table 1). Controls were matched to the speed skaters for sex and age to minimize the potential influence of these variables as independent factors. Exclusion criteria included a history of cardiovascular disease, chronic kidney disease, use of medications known to affect muscle markers (e.g., statins), and any serious injury or illness within three months prior to testing. To ensure the clear interpretation of ferritin levels, individuals receiving intravenous iron or therapeutic iron supplements were excluded. While the skaters received standard dietary guidance from a team nutritionist, no specific nutritional intervention program was implemented during the study.

Study Protocol

This study was conducted in February 2025, during the late competitive season for speed skating. Participants were instructed to refrain from strenuous physical activity for at least 24 hours prior to testing to minimize the acute effects of exercise. Measurements for speed skaters were conducted in the morning (9:00 am–12:00 pm) to ensure data were obtained in a resting state before daily training. This study included the following measured variables: subjective evaluations, including PFL, SQA, and IPAQ; physiological parameters such as pulse rate and blood pressure; and physical performance measures including TPA, thigh circumference, handgrip strength, and jump height (Figure 1).

Blood Markers

Serum concentrations of creatine kinase M-type CK (CK-M), Fer, pro-B-type natriuretic peptide (NT-proBNP), and

cardiac troponin T (cTnT) were analyzed using enzyme-linked immunosorbent assay (ELISA) kits and a microplate reader (450 nm; Varioskan LUX, Thermo Fisher Scientific, USA), with CK-M measured as mass concentration (ng/mL). The following commercial kits were used: CK-M (Human CKM SimpleStep ELISA Kit, ab264617; Abcam, Cambridge, UK), Fer (Human Ferritin ELISA Kit, RAB0197; RayBiotech, GA, USA), NT-proBNP (Human NT-proBNP ELISA Kit, EHPRONPPB; Thermo Fisher Scientific, MA, USA), and cTnT (Human Cardiac Troponin T ELISA Kit, EHTNNT1; Thermo Fisher Scientific). All samples were measured in duplicate, and the mean values were used for statistical analysis. Serum samples were diluted prior to CK-M analysis in accordance with the manufacturer's protocol. Because the present study targeted elite speed skaters, resting CK-M concentrations were expected to be high; however, commercially available ELISA kits with a wider validated analytical range were not available at the time of the study. As a result, although dilution reduced sample concentrations, some values remained above the upper limit of the calibrated standard curve (4.0 ng/mL) and were therefore estimated by extrapolation based on the fitted regression curve. These extrapolated values were treated as approximate estimates and were interpreted with caution, consistent with the exploratory nature of the analysis.

Blood samples were collected using serum collection tubes (200 μ L, No. 20.1291.0002; Microvette, clot activator and separator gel, Sarstedt AG & Co. KG, Nümbrecht, Germany). The blood samples were subsequently centrifuged at 1,500 \times g for 15 min to separate serum. Serum samples were stored at -80 $^{\circ}$ C until analysis. Prior to measurement, the samples were thawed at 4 $^{\circ}$ C in a refrigerator and then brought to room temperature (24 $^{\circ}$ C).

Subjective Assessment

Subjective evaluations were performed using visual analog scales, VASs, for the PFL and SQA (Q1, Figure 1). PFL and SQA were assessed using a 100-mm Visual Analog Scale (VAS) with defined anchors. For PFL, 0 mm was defined as “No fatigue” and 100 mm as “Exhaustion.” For SQA, 0 mm was defined as “Worst possible sleep” and 100

Table 2. Summary of subject profiles including years of competitive experience, athletic levels, and highest achievements.

Subject	Years of Experience	Competitive Level	Representative Performance
A	28	International level	5 th place, Olympic Games
B	21	International level	3 rd place, World Cup
C	20	International level	2 nd place, World Cup
D	16	International level	4 th place, World Cup
E	17	Top-50 nationally ranked	1 st , Japan Intercollegiate Championships
F	14	Top-50 nationally ranked	None
G	13	Japan Intercollegiate Championships	None
H	12	Japan Intercollegiate Championships	None
I	13	Japan Intercollegiate Championships	None

To protect personal information, the correspondence between individual participants and those listed in Table 1 is not disclosed. Anonymized performance levels (A–I) are provided for classification only and are not intended to correspond one-to-one with participants labeled S-1 to S-9.

mm as “Best possible sleep.” Participants were instructed to mark the point corresponding to their current state; the distance from the left anchor was measured in millimeters and divided by 10 to obtain a score ranging from 0 to 10. To assess physical activity, IPAQ was used (Q2). The IPAQ is a validated questionnaire designed to evaluate habitual physical activity over the past week and quantifies total physical activity (TPA), vigorous-intensity physical activity (VPA), moderate-intensity physical activity (MPA), and walking physical activity (WPA)¹³.

Physiological Measurements

Participants were seated in a resting position, and a wrist-type automatic blood pressure monitor (HEM-6231T; Omron Co., Kyoto, Japan) was used to measure pulse rate, diastolic blood pressure, DBP, and systolic blood pressure, SBP (M1, Figure 1). Mean arterial pressure, MAP, was calculated from DBP and SBP using the following equation¹⁴:

$$MAP = DBP + \frac{1}{3} (SBP - DBP) \quad (1)$$

Physical Performance Measurements

Physical activity was calculated based on weekly activity, in which walking, moderate-intensity activity, and vigorous-intensity activity were considered as 3.3, 4, and 8 METs, respectively; the total value was defined as TPA¹³. The distance between the upper border of the right patella and the anterior superior iliac spine was measured, and thigh circumference was measured at its midpoint (M2, Figure 1). Handgrip strength and jump height were used as the muscle strength (M3). Handgrip strength was measured bilaterally using a handgrip dynamometer (T-2177 Grip-D, Toei Light Co., Ltd., Saitama, Japan), and the mean value was used. Jump height was assessed using a countermovement jump test performed with the chalk jump method¹⁵.

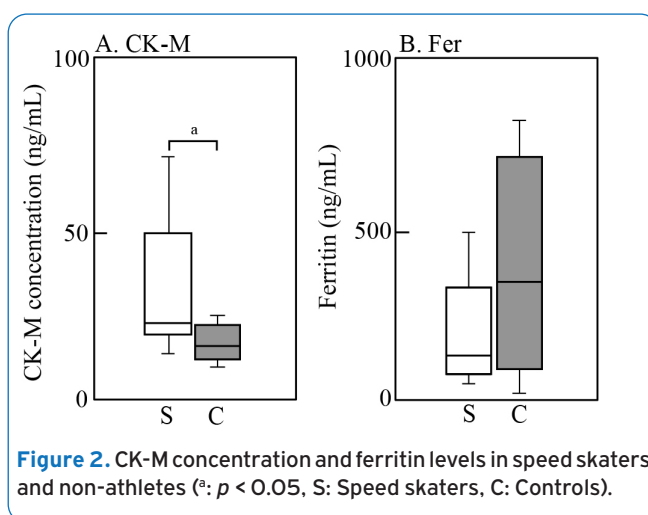


Figure 2. CK-M concentration and ferritin levels in speed skaters and non-athletes (^a: $p < 0.05$, S: Speed skaters, C: Controls).

Statistical Analysis

Given the small sample size, the multiple regression analysis was conducted as an exploratory, hypothesis-generating analysis rather than a confirmatory analysis. The use of stepwise variable selection and the limited sample size may reduce the robustness and generalizability of the estimated coefficients.

All statistical analyses were performed using the statistical package for the social sciences (SPSS version 29.0, IBM Corp., Armonk, NY, USA). Unless otherwise specified, measured values are shown as mean \pm standard deviation (SD), and the significance level was set at $p < 0.05$. Differences in measured variables between speed

skaters and controls were examined using the Mann-Whitney U test, and the rank-biserial correlation coefficient (γ_{rb}^*) was calculated to report effect sizes. Spearman's rank correlation coefficients (ρ) were calculated to assess relationships between two variables. Measurement values falling below the kit's limit of detection were coded as missing data without imputation. As a preliminary screening step, variables for which missing data exceeded 50% of the total sample (specifically, pro-BNP and cTnT) were excluded from all subsequent analyses to ensure reliability.

A multiple regression analysis was conducted with CK-M as the dependent variable and all other psychophysiological indicators as explanatory variables. Sex was considered as a potential covariate in the regression analyses; however, given the small sample size and its limited contribution to model performance, sex was not retained in the final regression models. The regression model for CK-M was constructed using a stepwise elimination procedure. Candidate variables were screened based on Spearman's rank correlation coefficients, excluding those highly correlated ($r > 0.7$) to avoid multicollinearity¹⁶. The selection process was repeated until the final regression model was obtained, in which all remaining variables showed F -values with $p < 0.1$.

Results

Athletic Characteristics

Among the speed skaters, four had participated in international competitions at the senior level, two were ranked within the top 50 in Japan, and three had competed in the all Japan intercollegiate championships (Table 2).

Biomarkers

Pro-BNP and cTnT were excluded from the statistical analyses because their concentrations were below the detection limit of 0.35 ng/mL in most participants. Figure 2 shows the comparisons of CK-M and Fer between the speed skaters and controls. The analytical range of the CK-M ELISA kit was 0.063 – 4.0 ng/mL. Although serum samples were diluted prior to analysis, some CK-M concentrations exceeded this range and were therefore estimated by extrapolation. CK-M levels were significantly higher in the speed skaters (32.6 ± 19.4 ng/mL) than in the controls (16.1 ± 5.1 ng/mL, $p = 0.031$). Although Fer levels were lower in the speed skaters (196.0 ± 145.7 ng/mL) than in the controls (393.6 ± 295.7 ng/mL), the difference was not statistically significant ($p = 0.260$).

Group comparison of input variables

Table 3 shows measured results of multiple psychophysiological characteristics including subjective assessments as PFL, SQA, and IPAQ. In addition to group comparisons, associations between CK-M levels and other variables were examined separately using Spearman's rank

Table 3. Participant characteristics, subjective assessments, PFL, SQA, and IPAQ.

	Speed skaters (n = 9)	Controls (n = 9)
Age (years)	24.0±4.3	24.3±4.3
Height (cm)	166.6±8.8	167.7±9.9
Body weight (kg)	67.7±7.6	61.9±11.6
Pulse rate (beats/min)	62.0±6.3	86.6 ±12.9
MAP (mmHg)	95.4±12.3	104.1±9.9
DBP (mmHg)	83.0±12.5	89.1±15.3
SBP (mmHg)	120.4±12.9	134.2±23.3
TPA (METs·min/week)	4116.0±2659.3	566.7±643.0
VPA (METs·min/week)	2097.8±1625.5	17.8±50.3
MPA (METs·min/week)	1151.1±843.4	266.7±328.8
WPA (METs·min/week)	867.2±938.3	282.3±573.7
Thigh circumference (cm)	59.2±3.0	51.2±4.9
Handgrip strength (kg)	38.7±10.6	32.1±7.4
Jump height (cm)	59.1±9.6	41.2±15.3
PFL (points)	3.1±0.7	3.9±0.7
SQA (points)	3.8±1.0	4.2±0.9
CK-M (ng/mL)	32.6±19.4	16.1±5.1
Ferritin (ng/mL)	196.0±145.7	393.6±295.7

Table 4. Group comparisons between speed skaters and controls.

	p value	γ_{rb}^*
Age (years)	0.893	-0.049
Height (cm)	1.000	-0.062
Body weight (kg)	0.387	0.259
Pulse rate (beats/min)	0.001	-0.901
MAP (mmHg)	0.143	-0.383
DBP (mmHg)	0.288	-0.309
SBP (mmHg)	0.200	-0.370
TPA (METs·min/week)	0.005	0.691
VPA (METs·min/week)	0.001	0.556
MPA (METs·min/week)	0.012	0.568
WPA (METs·min/week)	0.043	0.444
Thigh circumference (cm)	0.003	0.852
Handgrip strength (kg)	0.157	0.407
Jump height (cm)	0.030	0.617
PFL (points)	0.041	-0.593
SQA (points)	0.859	-0.099
CK-M (ng/mL)	0.032	0.605
Fer (ng/mL)	0.258	-0.333

p values derived from the Mann-Whitney U test comparing each group. γ_{rb}^* indicates the rank-biserial correlation coefficient as an effect size.

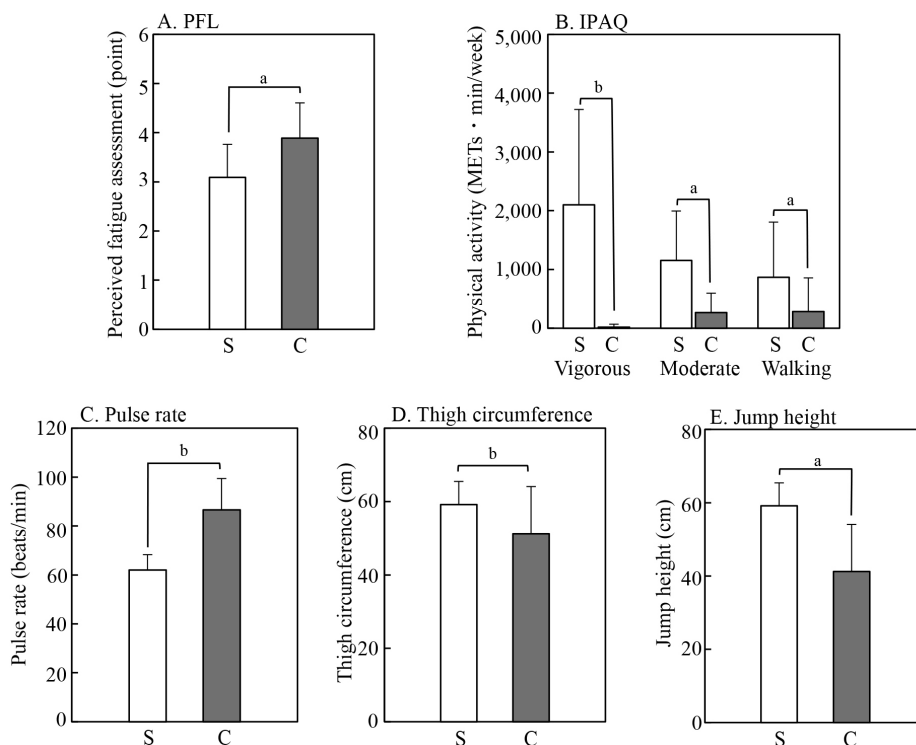


Figure 3. Comparison between speed skaters and non-athletes. (A) PFL. (B) Vigorous-, moderate-, and walking-IPAQ. (C) Pulse rate. (D) Thigh circumference. (E) Jump height. ^a: $p < 0.05$, ^b: $p < 0.01$. S: Speed skaters, C: Controls.

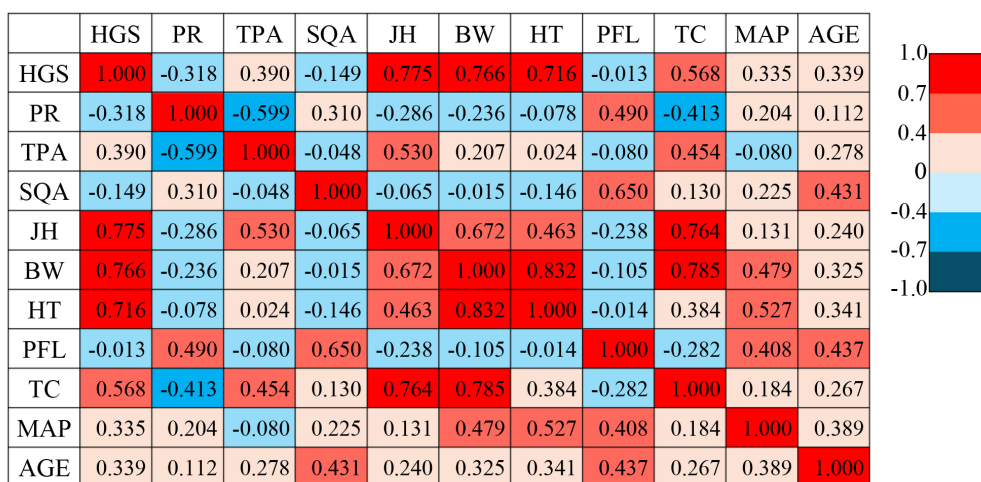


Figure 4. Heatmap of correlations among explanatory variables (HGS: Hand grip strength, PR: Pulse rate, TPA: Total physical activity, SQA: Sleep quality assessment, JH: Jump height, BW: Body weight, HT: Height, PFL: Perceived fatigue level, TC: Thigh circumference, MAP: Mean arterial pressure, AGE: Age).

Table 5. Results of multiple regression analyses predicting blood creatine kinase concentration.

	variable	B	SEB	β	p-value	95% CI	VIF
model 1	[Constant]	42.131					
$R^2 = 0.351$ $p = 0.010$	Pulse rate	-0.567	0.193	-0.593	0.010	[-0.97, -0.15]	1.000
model 2	[Constant]	54.086					
$R^2 = 0.591$ $p = 0.077$	HGS	0.157	0.476	0.100	0.748	[-0.89, 1.20]	2.494
	Pulse rate	-0.511	0.234	-0.534	0.052	[-1.02, 0.00]	1.610
	PFL	11.114	7.403	0.586	0.161	[-5.18, 27.4]	4.098
	TC	0.571	0.936	0.214	0.555	[-1.48, 2.63]	3.311
	MAP	-0.686	0.300	-0.543	0.043	[-1.34, -0.02]	1.514
	SQA	-5.851	5.606	-0.370	0.319	[-18.18, 6.48]	3.377
model 3	[Constant]	47.973					
$R^2 = 0.587$ $p = 0.038$	Pulse rate	-0.525	0.222	-0.549	0.035	[-1.00, -0.04]	1.558
	PFL	12.348	6.145	0.651	0.068	[-1.04, 25.73]	3.050
	TC	0.773	0.680	0.290	0.278	[-0.70, 2.25]	1.887
	MAP	-0.673	0.286	-0.532	0.037	[-1.29, -0.05]	1.486
	SQA	-6.877	4.483	-0.435	0.151	[-16.64, 2.89]	2.333
model 4	[Constant]	85.601					
$R^2 = 0.543$ $p = 0.028$	Pulse rate	-0.624	0.206	-0.652	0.010	[-1.06, -0.17]	1.316
	PFL	8.888	5.398	0.468	0.124	[-2.77, 20.54]	2.301
	MAP	-0.531	0.260	-0.420	0.062	[-1.09, 0.03]	1.203
	SQA	-4.299	3.910	-0.272	0.292	[-12.74, 4.14]	1.736
model 5	[Constant]	78.666					
$R^2 = 0.500$ $p = 0.018$	Pulse rate	-0.622	0.207	-0.649	0.010	[-1.06, -0.17]	1.316
	PFL	5.414	4.409	0.285	0.240	[-4.04, 14.86]	1.513
	MAP	-0.515	0.262	-0.407	0.069	[-1.07, 0.04]	1.199

correlation analysis. Significant correlations with CK-M were observed for pulse rate ($\rho = -0.561$, $p = 0.015$), TPA ($\rho = 0.483$, $p = 0.042$), VPA ($\rho = 0.627$, $p = 0.005$), and handgrip strength ($\rho = 0.584$, $p = 0.010$). Group comparisons of psychophysiological variables between speed skaters and controls are summarized in Table 4. IPAQ (Figure 3B), thigh circumference (Figure 3D), and jump height (Figure 3E) showed significantly higher values in the speed skaters than in the controls. PFL (Figure 3A) and pulse rate (Figure 3C) showed significantly lower values in the speed skaters than in the controls. SQA showed a slightly lower value in the speed skaters (3.8 ± 1.0 points) than in the controls (4.2 ± 0.9 points), although the difference was not statistically significant ($p = 0.859$). MAP was also slightly lower in the speed skaters (95.4 ± 12.3 mmHg) than in the controls (104.1 ± 9.9 mmHg), although this difference was not statistically significant ($p = 0.143$).

Multiple regression analysis

Collinearity was assessed by performing correlation analyses for all pairs of independent variables. Figure 4

shows a heatmap of the correlations among the independent variables. Because strong positive correlations ($r > 0.7$) were observed among height, body weight, handgrip strength, and jump height, these variables were excluded from the input variables.

Table 5 shows the results of the multiple regression analysis using a stepwise variable reduction method, summarizing the CK-M regression models with F values of $p < 0.1$. Among these, the regression model that included pulse rate, MAP, PFL, and SQA as explanatory variables (Model 4, $R^2 = 0.543$) was chosen as a more parsimonious model with acceptable explanatory power, rather than the one with the highest coefficients of determination.

$$\text{CK-M} = 85.601 - 0.652 \text{ Pulse rate} + 0.468 \text{ PFL} - 0.420 \text{ MAP} - 0.292 \text{ SQA} \quad (2)$$

As a comparison, we examined a simplified model using pulse rate as the sole explanatory (Model 1). This analysis confirmed a significant negative association ($\beta = -0.593$, $p = 0.010$, 95% CI [-0.97, -0.15]).

Figure 5 shows a forest plot of the estimated effects of the explanatory variables in Model 4. The F-value of

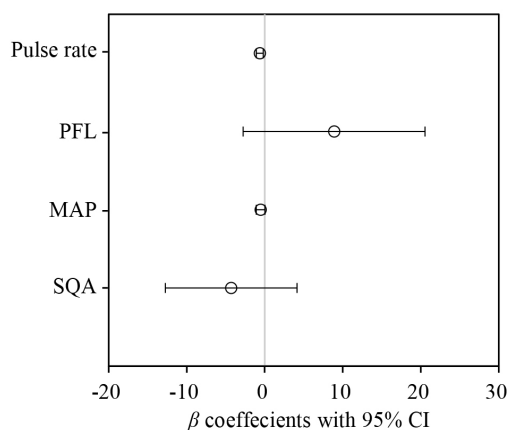


Figure 5. Forest plot of standardized β coefficients with 95% confidence intervals from multiple regression analysis (Model 4) explaining CK-M concentration ($R^2 = 0.543$, $p = 0.028$).

the CK-M regression model was significant ($p = 0.028$), with a coefficient of determination (R^2) of 0.543. All variance inflation factor (VIF) values were low, indicating no multicollinearity among the explanatory variables. The standardized regression coefficients indicated that the relative influence of the explanatory variables decreased in the following order: pulse rate, PFL, MAP, and SQA.

Discussion

The present study targeted top-level Japanese speed skaters, including international competitors, and attempted to construct a regression model of CK-M, a biomarker of muscle injury, based on combined information from multiple physical and psychological parameters.

CK-M levels were significantly higher in the speed skaters than in the controls (Figure 2A). Previous studies have reported injury-risk thresholds for CK expressed as enzymatic activity (U/L); although conversion between U/L and mass concentration (g/mL) is chemically feasible under standardized optimal temperature conditions, the present study quantified CK-M as mass concentration (ng/mL) using an ELISA-based assay, and therefore direct numerical comparison with activity-based thresholds was not performed. In speed skaters, an increased risk of injury has been reported when CK exceeds 467 U/L⁷, the risk of injury in skaters increases 2.56 times⁸. In addition, Kowalski et al. (2025) reported that CK levels were significantly higher after races than before in speed skaters¹¹. The present results are consistent with these findings¹¹, suggesting that elevated resting CK levels in athletes may reflect muscle injury caused by training.

In contrast, no significant difference in Fer levels was

observed between the two groups (Figure 2B). In the field of sports science, Fer has been investigated as a potential indicator for detecting iron deficiency related fatigue caused by endurance exercise^{19,20}. Although speed skating is characterized by explosive lower-limb power, athletes engage in both aerobic and high-intensity training throughout the year²¹⁻²³, which may predispose them to chronic iron deficiency²⁴. The absence of a significant difference in Fer levels between the groups in the present study may be attributed to adequate nutritional management among the skaters at the time of measurement. To verify this interpretation, future studies should include detailed dietary assessments in conjunction with biomarker analyses.

Thigh circumference and jump height were significantly higher in the speed skaters than in the controls (Figure 3D and E), whereas PFL was significantly lower in the speed skaters (Figure 3A). A previous study reported a correlation between jump height performance and 500 m race time²⁵. These variables are considered to reflect muscular strength and performance, confirming the appropriateness of participant selection among the speed skaters in this study.

Before interpreting the results of the multiple regression analysis, several important limitations should be acknowledged. Given the small sample size and the use of stepwise variable selection, the regression models are prone to overfitting, and the estimated coefficients and coefficient of determination may be unstable. Accordingly, the regression analysis should be interpreted as exploratory and hypothesis-generating rather than confirmatory. Given the limited sample size relative to the number of variables examined, overfitting and multiple comparisons cannot be excluded, and the results therefore require confirmation in larger, independent cohorts. The exploratory multiple regression analysis identified a model including pulse rate, MAP, PFL, and SQA as explanatory variables, which was selected as a more parsimonious model with acceptable explanatory power in consideration of the small sample size and the risk of overfitting. VIF values ranged from 1.1 to 2.4, indicating no multicollinearity (Table 5). Among the psychophysiological parameters that showed significant differences between the two groups, pulse rate and PFL were selected in the regression model. The exclusion of physical activity, thigh circumference, and jump height as explanatory variables may reflect overlapping information with pulse rate and other correlated performance variables. In this study, the speed skaters showed lower values for pulse rate, MAP, PFL, and SQA than the controls (Table 3). Previous studies have reported that endurance training lowers pulse rate and MAP^{26,27} and that individuals with greater muscle strength tend to exhibit lower PFL²⁸. Conversely, high-intensity training has been shown to improve muscle strength but reduce sleep quality²⁹. Given the limited sample size and the risk of overfitting in multivariable models, we also examined a simplified univariate model using pulse rate alone. The

consistent negative association observed in this model supports the robustness of the relationship between resting pulse rate and CK-M levels, while emphasizing that this finding should be interpreted as exploratory. These findings suggested that the observed associations between pulse rate, MAP, PFL, and CK-M may reflect underlying physiological relationships, although the exploratory nature of the analysis and the small sample size warrant cautious interpretation.

Conclusions

This study examined both speed skaters and non-athletes, by measuring not only blood biomarkers related to sports injury, such as blood creatine kinase and ferritin, but also various noninvasive physical and subjective parameters, to analyze their interrelationships and to construct a regression model for CK-M. As a result, the present analysis suggested that CK-M levels may be associated with a combination of pulse rate and mean arterial pressure, together with physical fatigue level and subjective quality assessment, among the multiple psychophysiological parameters conventionally used for athlete evaluation. Taken together, the present findings suggest that resting CK-M levels may be associated with several routinely assessed psychophysiological parameters in elite speed skaters. These preliminary observations highlight patterns that merit confirmation in larger, longitudinal cohorts, rather than providing evidence for clinical prediction or decision thresholds.

The sample size in this study was relatively small ($n = 18$), and the sample-per-variable of the constructed model was below 10. However, the participants included high-level speed skaters, some of whom had experience competing in senior international events; therefore, this limitation was considered unavoidable in obtaining academically valuable data.

Ethics approval

The study protocol was approved by the ethics committee of the Shinshu University School of Medicine (Approval No. 6360).

Consent to participate

Prior to participation, all subjects were informed of the purpose and procedures of the study both verbally and in writing, and written informed consent was obtained from each participant.

Author's Contributions

Masaki Yamaguchi was responsible for the conception and design of the study. The recruitment of participants was conducted by Masahiro Yuki and Takanori Matsuura. Blood sampling was carried out by Koji Yoshie and Yukari Okuma. Koichiro Kuwahara provided medical advice and supervision. Data acquisition was performed by Takanori Matsuura and Masaki Yamaguchi. Data analysis and interpretation were conducted by Takanori Matsuura. The initial draft of the manuscript was prepared by Takanori Matsuura, and the manuscript was reviewed and proofread by Masaki Yamaguchi and Masahiro Yuki.

All authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work.

Funding

This work was supported by the Keirin Autorace Foundation, Japan (No. 2024M-418, PI: Masaki Yamaguchi), for the project Development of a Wearable Sensor for Detecting Muscle Fatigue and Anemia to Prevent Sports Injuries.

References

1. Shigematsu R, Katoh S, Suzuki K, Nakata Y, Sasai H. Sports specialization and sports-related injuries in Japanese school-aged children and adolescents: A retrospective descriptive study. *Int. J. Environ. Res. Public Health.* 2021;18:7369.
2. Jayanthi N, Schley S, Cumming SP, Myer GD, Saffel H, Hartwig T, Gabbett T. Developmental training model for the sport specialized youth athlete: a dynamic strategy for individualizing load-response during maturation. *Sports health* 2022;14(1):142-53.
3. Um TT, Babakeshizadeh V, Kulić D. Exercise Motion Classification from Large-Scale Wearable Sensor Data Using Convolutional Neural Networks. *arXiv preprint arXiv 2016;1610.07031.*
4. Buchheit M. Monitoring training status with HR measures: do all roads lead to Rome? *Front Physiol* 2014;5:73.
5. Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. *ClinChem Lab Med* 2010;48(6):757-767.
6. Lee EC, Fragala MS, Kavouras SA, Queen RM, Pryor J, Casa DJ. Biomarkers in Sports and Exercise: Tracking Health, Performance, and Recovery in Athletes. *J Strength Cond Res* 2017;31(10):2920-2937.
7. Dupuy O, Douzi W, Theurot D, Bosquet L, Dugué B. An Evidence-Based Approach for Choosing Post-exercise Recovery Techniques to Reduce Markers of Muscle Damage, Soreness, Fatigue, and Inflammation: A Systematic Review With Meta-Analysis. *Front Physiol* 2018;9:403.
8. Rubeor A, Goojha C, Manning J, White J. Does Iron Supplementation Improve Performance in Iron-Deficient Nonanemic Athletes? *Sports Health* 2018;10(5):400-405.
9. Bok D, Jukić I. Muscle Damage During a Soccer World Cup Preparatory and Competition Period. *Int J Sports Physiol Perform* 2019;15(4):496-502.
10. Ferrera A, Gioia GD, Spinelli A, Fiore R, Zampaglione D, Serdoz A, Squeo MR. Blood test findings in a large cohort of Olympic athletes. *J Sci Med Sport* 2025.
11. Kowalski T, Rębiś K, Malczewska-Lenczowska J, Klusiewicz A, Starczewski M, Klich S, Kasiak P. Optimizing Recovery Strategies in Elite Speedskating: A Comparative Analysis of Different Modalities. *J Funct Morphol Kinesiol* 2025;10(1):34.
12. Wang F, Cui J, Wu Y, Chen H, Hong P, Xue L. Research

- on Injury Risk of Elite Male Athletes in Racing Ice Sports based on Blood Indexes. *Int J Sports Med* 2025;46(11):840-849.
13. Craig CL, Marshall AL, Sjöström M. International physical activity questionnaire. 12-country reliability and validity. *Med Sci Sports Exerc* 2003;35:1381-1395.
 14. Gauer O. Kreislauf des Blutes. In: Landois L, editor. *Lehrbuch der Physiologie des Menschen*. Berlin: Urban and Schwarzenberg; 1960.
 15. Van Dalen D. New studies in the Sargent jump. *Res Q Am Assoc Health Phys Educ Recreat* 1940;11(2):112-115.
 16. Yamaguchi M, Okamura S, Yamaji T, Iwasaki M, Tsugane S, Shetty V, Koizumi T. Plasma cytokine levels and the presence of colorectal cancer. *PLOS ONE* 2019;14(3):e0213602.
 17. Hong JH, Kim HJ, Ki, KJ, Suzuki K, Lee IS. Comparison of Metabolic Substrates between Exercise and Cold Exposure in Skaters. *J Physiol Anthropol* 2008;27:273-281.
 18. Hartmann U, Mester J. Training and overtraining markers in selected sport events. *Med Sci Sports Exerc* 2000;32:209-215.
 19. Pedlar CR, Bruignara C, Bruinvels G, Burden R. Iron balance and iron supplementation for the female athlete: a practical approach. *Eur J Sport Sci* 2018;18(2):295-305.
 20. Kardasis W, Naquin ER, Garg R, Arun T, Gopianand JS, Karmakar E, Gnana-Prakasam JP. The IRONY in Athletic Performance. *Nutrients* 2023;15:4945.
 21. Orié J, Hofman N, de Koning JJ, Foster C. Thirty-eight years of training distribution in Olympic speed skaters. *Int J Sports Physiol Perform* 2014;9:93-99.
 22. Orié J, Hofman N, Meerhoff LA, Knobbe A. Training distribution in 1500-m speed skating. A case study of an olympic gold medalist. *Int. J. Sports Physiol. Perform* 2021;16:149-153.
 23. Sandbakk Ø, Tønnessen E, Sandbakk SB, Losnegard T, Seiler S, Haugen T. Best-practice training characteristics within olympic endurance sports as described by norwegian world-class coaches. *Sport Med Open*. 2025;11:45.
 24. Mayr A, Kuipers H, Falk M, Santer P, Wierer B. Comparison of hematologic data in elite junior speed skaters and in non-athletic juniors. *Int J Sports Med* 2006;27:283-288.
 25. Zukowski M, Herzog W, Jourdan MJ. Velocity-Load Jump Testing Predicts Acceleration Performance in Elite Speed Skaters: But Does Movement Specificity Matter?. *Int J Sports Physiol Perform* 2024;19(8):757-764.
 26. Wilmore JH, Stanforth P, Gagnon J, Leon AS, Skinner JS, Bouchard C. Endurance exercise training has a minimal effect on resting heart rate: the HERITAGE study. *Med Sci Sports Exerc* 1996;28(7):829-835.
 27. Cornelissen VA, Smart NA. Exercise training for Blood Pressure: A systematic review and meta-analysis. *Journal of the American Heart Association* 2013;2(1):e004473.
 28. Demura S, Yamada T, Shimada S, Uchiyama M. Influence of exercise habits and physical fitness level on subjective fatigue symptoms in adolescent students. *Health* 2011;3:20-25.
 29. Kong Z, Wei X, Shen M, Cheng Y, Feng J. Interval training has more negative effects on sleep in adolescent speed skaters: a randomized cross controlled trial. *Frontiers in Sports and Active Living* 2024;6.