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Original Article

Dynamics of urinary transferrin, KIM-1, RBP, MCP-1 and NGAL following 5-km running exercise in a sub-plateau environment and their associations with renal function parameters

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Abstract: Objective: This study aimed to investigate the dynamic changes in urinary transferrin, kidney injury molecule-1 (KIM-1), retinol-binding protein (RBP), monocyte chemoattractant protein-1 (MCP-1), and neutrophil gelatinase-associated lipocalin (NGAL) before and after 5-km running exercise in a sub-plateau environment, and to analyze their associations with estimated glomerular filtration rate (eGFR) and albumin-to-creatinine ratio (ACR). Methods: Blood and urine samples were collected from healthy male participants who completed 5-km running exercise in a sub-plateau environment, at five time points: pre-training (baseline), and 0.5, 12, 24, and 48 hours post-training. Urinary biomarker concentrations were measured and normalized to creatinine (Cr); serum creatinine (Scr) and urinary albumin were also measured to calculate eGFR and ACR. Results: Following training, Scr peaked at 12 h post-exercise, while eGFR reached its nadir at the same time point. All urinary biomarkers (ACR, transferrin, RBP, NGAL, MCP-1, KIM-1) showed significant temporal variations (all $P<0.001$), generally peaking at 12 h post-training and returning toward baseline by 48 h. At 12 h post-training, all biomarkers except KIM-1 showed significant positive correlations with log-transformed ACR (logACR). Hierarchical regression analysis revealed that NGAL/Cr ($\beta = 0.453$, $P<0.001$), MCP-1/Cr ($\beta = 0.707$, $P<0.001$), and transferrin/Cr ($\beta = 0.611$, $P<0.001$) were independently associated with increased logACR. Stepwise regression showed that MCP-1/Cr was the only variable retained for predicting eGFR ($\beta = 0.162$, $P = 0.064$). Conclusion: Sub-plateau high-intensity training induces transient, reversible increases in urinary NGAL, MCP-1, transferrin, and RBP, and show a trend toward an association with eGFR and ACR elevation. NGAL and MCP-1 show stable, independent associations with both functional indicators, suggesting their utility as reliable biomarkers for early renal injury under combined hypoxia-exercise stress.

Keywords: Submaximal exercise, acute renal stress, urinary biomarkers, transferrin, KIM-1, RBP, MCP-1, NGAL

Introduction

High-intensity training in sub-plateau environments (altitude usually ranging from 1500 to 2500 meters) combines hypobaric hypoxia with strenuous physical exertion, potentially imposing unique stressors on the kidney. The kidney, being highly sensitive to hemodynamic fluctuations, oxidative stress, and inflammatory responses, may sustain subclinical injury that is not readily detectable by conventional markers. While exercise-induced proteinuria and transient serum creatinine (Scr) elevations are well-documented, the early renal responses to the synergistic stress of hypoxia and intense exercise remain incompletely understood [1]. Understanding these physiological responses may contribute to the evaluation of exercise-associated renal stress and the development of appropriate monitoring strategies for individuals performing strenuous physical activity in sub-plateau environments.

However, in clinical practice, acute kidney injury (AKI) is still mainly diagnosed on the basis of serum creatinine and urine output, two markers

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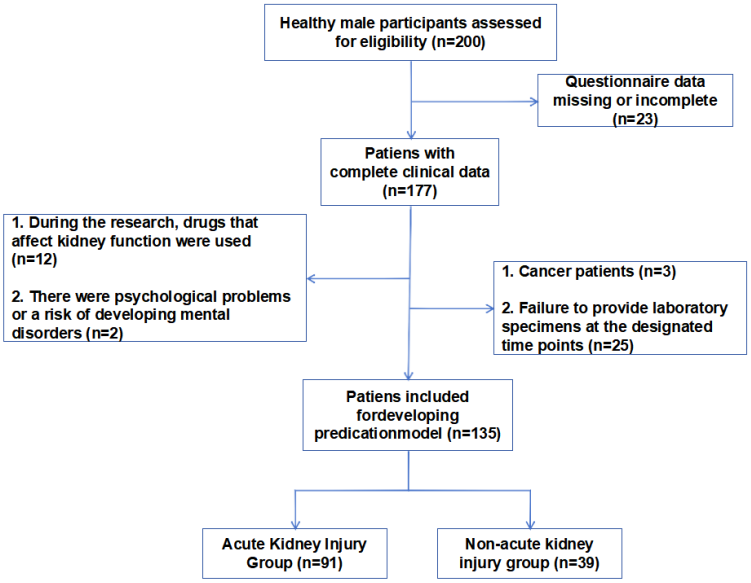


Figure 1. Flowchart for participant screening.

that lack sufficient sensitivity for detecting early, particularly structural and tubular, damage [2]. This limitation has fueled growing enthusiasm for the discovery of novel urinary markers that reflect different pathophysiologic domains: transferrin for glomerular health, retinol-binding protein (RBP) for proximal tubule reabsorption, kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) for tubule injury, and monocyte chemotactic protein-1 (MCP-1) for renal inflammation [3-6]. These biomarkers have shown promise in several models of AKI [7, 8], and appear elevated after strenuous exercise or in chronic hypoxia [9, 10]. However, in the specific setting of sub-plateau, at least three critical questions remain unresolved: (1) What is the time course over which these biomarkers appear and peak during the acute post-exercise period (0-48 h)? (2) What chronological sequence exists among markers of different injury processes? and (3) What is the concordance between these biomarkers and classic functional indices of kidney function, including the estimated glomerular filtration rate (eGFR) and the albumin-to-creatinine ratio (ACR)?

To this end, this retrospective observational study systematically evaluates the effect of a 48 h high-intensity 5-km running exercise in a sub-plateau environment on subclinical kidney injury in healthy male participants. By observing the dynamic changes in urinary KIM-1, NGAL, RBP, MCP-1 and transferrin at different time points and correlation with eGFR and ACR, this study may provide a scientific basis for early risk assessment and the development of kidney protection strategies for healthy male individuals undergoing high-intensity running exercise.

Methods

Study population

This study adopted a retrospective observational design. In May 2025, 135 healthy male participants who underwent 5-km running exercise in an alpine area of Yunnan Province were enrolled as participants (**Figure 1**). The study protocol was approved by the Ethics Committee of the 920th Hospital of the Joint Logistics Support Force of Kunming, Yunnan Province (Ethics Approval No.: 920IEC/AF/61/2021-01.1). All participants provided written informed consent after receiving detailed information about the purpose and process of this study. The inclusion criteria for this study were as follows: (1) Age of 19 to 37 years; (2) Normal Scr and urine routine test results were normal before training, with eGFR >60 mL/min/1.73 m^2 [11]; (3) No history of hypertension, diabetes, or cardiovascular disease; (4) No use of non-steroidal anti-inflammatory drugs, glucocorticoids, or other medications affecting renal function within the preceding 1 month; (5) No history of fever, diarrhea, or trauma within one week before training. Exclusion criteria were: (1) Use of medications that affect renal function during the study; (2) Presence of psychological issues or a predisposition to mental illness; (3) Other causes of renal impairment, including bilateral cortical necrosis, vasculitis, interstitial nephropathy, pyelonephritis, malignant hypertension, glomerulonephritis, and amyloidosis; (4) Diagnosis of renal disease; (5) Missing or incomplete questionnaire data; (6) Failure to provide laboratory specimens at the designated time points.

Training programs and grouping

This research was conducted from May 1 to 31, 2025, at a study site in a sub-plateau region of Yunnan Province. The training area was at an altitude of 1600-2000 meters, with an estimated barometric pressure of 596-632 mmHg and an ambient partial pressure of oxygen (PO_2) of approximately 125-132 mmHg, representing a moderate hypobaric hypoxic environment characterized by large day-night temperature differences and high relative humidity. During the training period, the average morning environmental temperature was 17 ± 2°C, the average daytime temperature was 25 ± 3°C, and the relative humidity was 75.5 ± 3.6%. The participants completed a standardized 5-km running exercise protocol. Exercise sessions were scheduled at 3:00 PM. Meals and water were provided uniformly during the training period, and no nutritional supplements were used. Following training, AKI was diagnosed according to the Kidney Disease: Improving Global Outcomes (KDIGO) AKI diagnostic criteria, using the pre-training baseline Scr as a reference. AKI was defined as an increase in Scr of ≥26.5 μmol/L (0.3 mg/dL) or ≥50% from baseline within 48 h after training. Participants who did not meet these criteria were classified into the non-AKI group [12].

Sample collection and processing

Blood and urine samples were collected at the following five time points: pre-training (baseline), and at 30 min, 12 h, 24 h, and 48 h post-training. Venous blood samples (10 mL) were collected into vacuum blood collection tubes containing a coagulant (BD Vacutainer SST, Franklin Lakes, NJ, USA). After standing at room temperature for 30 min, the tubes were centrifuged at 3000 rpm for 15 min at 4°C. The separated serum was aliquoted into 1.5 mL cryovials and stored at -80°C until analysis. Urine samples (15 mL) were collected into urine collection cups and immediately centrifuge at 3000 rpm for 10 min at 4°C. The supernatant was transferred to 1.5 mL cryovials and stored at -80°C until testing. All samples were centrifuged within 2 h of collection.

Biomarker testing

Scr, urinary creatinine, and urinary albumin were measured using an RXDaytona clinical chemistry analyzer. Systolic and diastolic blood pressure were measured three times in a resting state using an electronic sphygmomanometer, and the average values were recorded. Urinary protein was assessed by calculating the ACR in mg/g. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [13]. The levels of urinary transferrin, RBP, and NGAL, were measured by solid-phase enzyme-linked immunosorbent assay (ELISA) (Thermo Fisher Scientific). Urinary KIM-1 and MCP-1 were detected using ELISA kits from Shanghai Enzyme-Linked Biotechnology Co., Ltd. All urinary biomarkers were normalized to urinary creatinine concentration.

Statistical analysis

Data were statistically analyzed using SPSS 25.0. For continuous variables, the Shapiro-Wilk test was used to assess normality. Variables meeting the normal distribution were expressed as mean ± standard deviation ($\overline{x}$ ±*sd*), while those with a skewed distribution were presented as median (interquartile range) [M (IQR)]. Categorical variables were expressed as frequency (percentage) [n (%)]. Comparisons between groups were conducted using independent sample t-tests or Mann-Whitney U tests (for two groups), and either one-way ANOVA or Kruskal-Wallis H tests were used for comparisons among multiple groups. Overall differences in urinary marker levels across time points were evaluated using the Friedman M test, and pairwise post-hoc comparisons were performed using Dunn's test. Bonferroni correction was applied for multiple comparisons. Spearman rank correlation analysis was used to assess correlations between each urinary biomarker (after creatinine correction) and eGFR and ACR. In multivariate linear regression analysis, eGFR and log-transformed ACR (logACR) were the dependent variables, and urinary biomarkers were the independent variables. Stepwise regression was applied for eGFR, and hierarchical regression was applied for logACR. Covariates included age, body mass index (BMI), systolic blood pressure, training intensity, and eGFR. All regression models underwent multicollinearity diagnosis (variance inflation factor [VIF] >10 considered indicative of multicollinearity), and skewed urine markers were log-transformed before being entered into the models. Receiver operating characteristic

Table 1. Comparison of baseline characteristics between AKI group and non-AKI group subjects

Parameters	Overall (n = 135)	AKI group (n = 34)	Non-AKI group (n = 101)	$t/Z/\chi^2$	p
Age, years	23.00 (21.00-24.00)	22.50 (21.00-24.75)	23.00 (21.00-24.00)	0.553	0.577
BMI, kg/m^2	22.33 (22.03-22.77)	22.16 (21.89-22.41)	22.38 (22.06-22.79)	-1.959	0.050
Exercise frequency (≥2 times/day)	47 (34.8%)	8 (23.5%)	39 (38.6%)	3.030	0.220
Sleep duration (7-8 h)	96 (71.1%)	23 (67.6%)	73 (72.3%)	0.088	0.767
Sleep quality (ordinary)	46 (34.1%)	12 (35.3%)	34 (33.7%)	0.063	0.969
Smoking history (yes)	69 (51.1%)	17 (50.0%)	52 (51.5%)	0.000	1.000
SBP, mmHg	117.81 ± 4.09	117.32 ± 4.58	117.97 ± 3.93	-0.795	0.428
DBP, mmHg	75.24 ± 3.30	74.32 ± 3.25	75.55 ± 3.27	-1.902	0.059
Scr (μmol/L)	80.66 ± 4.83	82.14 ± 4.56	80.17 ± 4.84	2.083	0.039
eGFR (mL/min/1.73 m^2)	118.32 (113.03-121.83)	116.00 (110.64-121.00)	119.26 (113.62-121.98)	-1.977	0.048
ACR (mg/g Cr)	3.39 (2.47-4.80)	3.05 (2.37-4.09)	3.49 (2.50-4.93)	-1.252	0.211
TRF/Cr (μg/g Cr)	0.50 ± 0.06	0.50 ± 0.06	0.49 ± 0.06	0.738	0.462
NGAL/Cr (ng/g Cr)	0.11 ± 0.04	0.10 ± 0.05	0.11 ± 0.04	-1.099	0.274
RBP/Cr (μg/g Cr)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.232	0.817
MCP-1/Cr (pg/mg Cr)	0.69 ± 0.16	0.65 ± 0.14	0.70 ± 0.16	-1.592	0.114
KIM-1/Cr (pg/mg Cr)	1.79 ± 0.30	1.80 ± 0.26	1.78 ± 0.31	0.263	0.793

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; ACR, albumin-to-creatinine ratio; TRF/Cr, transferrin-to-creatinine ratio; NGAL/Cr, neutrophil gelatinase-associated lipocalin-to-creatinine ratio; RBP/Cr, retinol-binding protein-to-creatinine ratio; MCP-1/Cr, monocyte chemoattractant protein 1-to-creatinine ratio; KIM-1/Cr, kidney injury molecule 1-to-creatinine ratio.

(ROC) curves were constructed to assess the predictive ability of 12 h post-training urinary biomarkers transferrin (TRF)/Cr, NGAL/Cr, RBP/Cr, MCP-1/Cr and KIM-1/Cr for AKI. The area under the curve (AUC) with 95% confidence interval (CI) was calculated for each biomarker. The optimal cut-off value was determined by maximizing the Youden index, and the corresponding sensitivity and specificity were reported. Differences between AUCs were compared using the DeLong test. A two-sided P<0.05 was considered statistically significant.

Result

Analysis of baseline data of the subjects

A total of 135 participants were enrolled, of whom 34 were in the AKI group and 101 in the non-AKI group. As shown in **Table 1**, most baseline characteristics were comparable between the two groups. Specifically, there were no significant differences in age, BMI, exercise frequency, sleep duration, sleep quality, smoking history, systolic blood pressure (SBP), and diastolic blood pressure (DBP) (all P>0.05). Significant differences were observed in kidney function parameters: participants in the AKI group exhibited higher Scr levels (82.14 ± 4.56 vs. 80.17 ± 4.84 μmol/L, P = 0.039) and lower eGFR values (116.00 [110.64-121.00] vs. 119.26 [113.62-121.98] mL/min/1.73 m^2, P = 0.048) compared to the non-AKI group. Although the difference in BMI approached statistical significance (P = 0.05), none of the baseline urinary biomarkers, including transferrin, NGAL, RBP, MCP-1, and KIM-1, differed significantly between the groups (all P>0.05).

The changing trends of urine markers at different time points after training

The temporal changes in renal function and urinary biomarkers before and after 5-km running exercise are summarized in **Figure 2**. All measured parameters showed significant temporal variations across the five time points (all P<0.001). Scr levels increased rapidly within 30 min post-training, peaked at 12 hours (96.82 [89.98-109.81] μmol/L), and returned to baseline by 48 hours. Conversely, eGFR demonstrated a reciprocal pattern, reaching its nadir at 12 hours (95.22 [81.26-103.41] mL/min/1.73 m^2) before recovering to baseline. All urinary biomarkers showed a significant post-exercise surge. Urinary ACR rose sharply at 30 min and then gradually decreased. Markers of tubular injury, including TRF/Cr, NGAL/Cr, MCP-1/Cr, and KIM-1/Cr showed a delayed peak at 12 hours, with KIM-1/Cr exhibiting the greatest fold change from baseline. Urinary RBP/Cr showed only a moderate increase. At 48 h post-training, most of the biomarkers significantly decreased and approached baseline levels, indicating that the intense exercise pro-

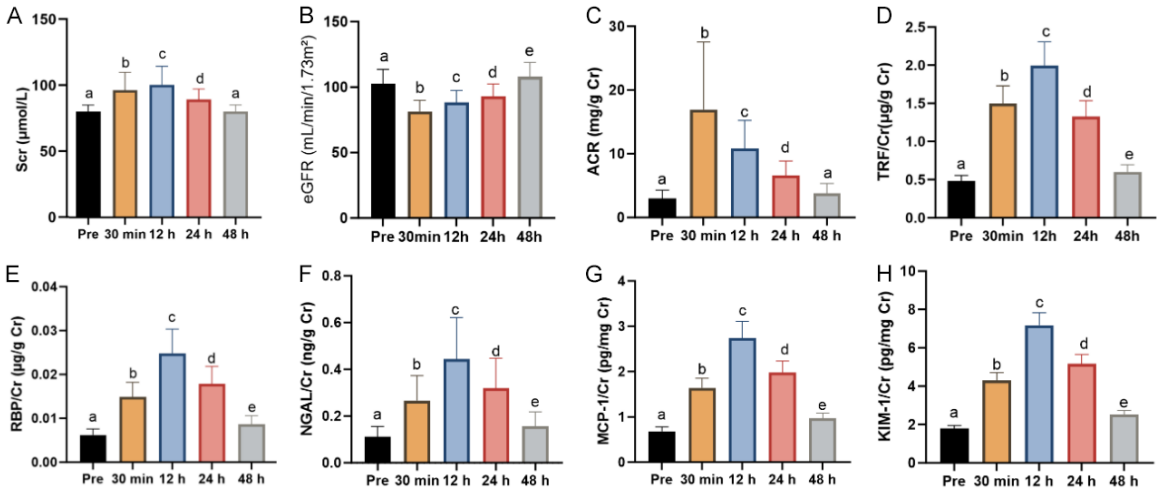


Figure 2. Temporal profiles of renal function and urinary biomarkers following sub-plateau training. (A) Serum creatinine (Scr), (B) estimated glomerular filtration rate (eGFR), (C) urinary albumin-to-creatinine ratio (ACR), (D) urinary biomarkers normalized to creatinine: transferrin (TRF/Cr), (E) retinol-binding protein (RBP/Cr), (F) neutrophil gelatinase-associated lipocalin (NGAL/Cr), (G) monocyte chemotactic protein-1 (MCP-1/Cr), (H) kidney injury molecule-1 (KIM-1/Cr). Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Different letters (a, b, c, d, e) above the bars indicate significant differences between the groups ($P<0.05$), whereas sharing the same letter indicates no significant difference ($P>0.05$).

Table 2. Correlation analysis of urine markers and renal function indicators 12 hours after training

Biomarker	eGFR		logACR	
	r	p	r	p
TRF/Cr (μg/g Cr)	-0.078	0.368	0.546	<0.001
NGAL/Cr (ng/g Cr)	-0.056	0.518	0.439	<0.001
RBP/Cr (μg/g Cr)	-0.030	0.731	0.306	<0.001
MCP-1/Cr (pg/mg Cr)	0.127	0.142	0.678	<0.001
KIM-1/Cr (pg/mg Cr)	0.054	0.530	-0.071	0.414

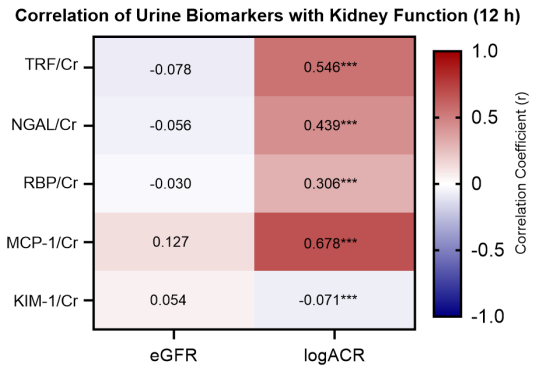


Figure 3. Spearman correlation heatmap between urinary biomarkers and renal function parameters at 12 h post-training. Color intensity and numerical values represent Spearman correlation coefficients. logACR, log-transformed albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate. ***P<0.001.

duced a transient and reversible renal response.

Correlation analysis of urine markers with eGFR and ACR

Correlation analyses between urinary biomarkers and renal function parameters at 12 hours post-training are presented in **Table 2** and **Figure 3**. All urinary biomarkers, with the exception of KIM-1/Cr, demonstrated significant positive correlations with logACR, a marker of glomerular injury. Specifically, MCP-1/Cr showed the strongest correlation with logACR ($r = 0.678$, $P<0.001$), followed by TRF/Cr ($r = 0.546$, $P<0.001$), NGAL/Cr ($r = 0.439$, $P< 0.001$), and RBP/Cr ($r = 0.306$, $P<0.001$). In contrast, none of the urinary biomarkers exhibited significant correlations with eGFR at this time point (all $P>0.05$).

Regression analysis of influencing factors

To identify independent predictors of eGFR at 12 hours post-training, a stepwise multiple linear regression analysis was performed. Candidate variables included age, BMI, baseline systolic and diastolic blood pressure, and the

Table 3. Stepwise regression analysis of factors affecting eGFR level 12 hours after training

Model	Biomarker	Standard β	t	P
1	MCP-1/Cr (pg/mg Cr)	0.162	1.871	0.064

Note: Age, BMI, blood pressure and other urine markers were all excluded during the stepwise regression screening process.

Table 4. Stratified regression model analysis of urine markers for predicting logACR levels

Biomarker	Model	Standard β	t	P
NGAL/Cr (ng/g Cr)	1	0.469	6.095	<0.001
	2	0.452	5.794	<0.001
	3	0.453	5.772	<0.001
MCP-1/Cr (pg/mg Cr)	1	0.709	11.602	<0.001
	2	0.699	11.231	<0.001
	3	0.707	11.265	<0.001
TRF/Cr (μg/g Cr)	1	0.611	8.875	<0.001
	2	0.599	8.524	<0.001
	3	0.611	8.634	<0.001
RBP/Cr (μg/g Cr)	1	0.325	3.985	<0.001
	2	0.305	3.692	<0.001
	3	0.309	3.715	<0.001

Note: Model 1 adjusts for age and BMI; Model 2 adjusts the baseline systolic blood pressure and baseline diastolic blood pressure based on Model 1; Model 3 adjusts the 12-hour eGFR based on Model 2.

five urinary biomarkers measured at 12 hours (TRF/Cr, NGAL/Cr, RBP/Cr, MCP-1/Cr, and KIM-1/Cr). The entry and removal criteria were set at $\alpha = 0.15$ and $\alpha = 0.20$, respectively. As shown in **Table 3**, MCP-1/Cr was the sole variable retained in the final model, demonstrating a trend toward a positive association with eGFR levels at 12 hours (standardized $\beta = 0.162$, $t = 1.871$, $P = 0.064$). All other candidate variables, including traditional clinical parameters and the remaining urinary biomarkers, were excluded during the stepwise selection process. These findings suggest that among the evaluated markers, MCP-1/Cr may explain post-training eGFR fluctuations most strongly, independent of demographic and hemodynamic confounders.

To evaluate the independence of the associations between urinary biomarkers and glomerular filtration barrier injury (log-transformed ACR), a series of hierarchical linear regression models were constructed (**Table 4**). The results demonstrated robust and consistent associations across all three hierarchical models. NGAL/Cr, MCP-1/Cr, and TRF/Cr each exhibited strong positive associations with logACR, with standardized beta coefficients remaining largely unchanged after sequential adjustment for demographic, hemodynamic, and renal function covariates. Specifically, in the fully adjusted Model 3, the standardized beta coefficients were 0.453 for NGAL/Cr ($P<0.001$), 0.707 for MCP-1/Cr ($P<0.001$), and 0.611 for TRF/Cr ($P<0.001$). In contrast, RBP/Cr showed a modest but statistically significant association ($\beta = 0.309$, $P< 0.001$), whereas KIM-1/Cr did not demonstrate a significant association with logACR.

Predictive performance of 12-h urinary biomarkers for AKI

To determine which urinary biomarker best identified participants who subsequently met KDIGO creatinine-based AKI criteria, ROC curve analyses were performed on the 12-h post-training measurements. The ROC curves are presented in **Figure 4**, and the detailed diagnostic performance metrics are summarized in **Table 5**. None of the five urinary biomarkers demonstrated strong discriminative power for predicting KDIGO-defined AKI in this cohort. MCP-1/Cr yielded the highest AUC at 0.596 (95% CI 0.483-0.710), followed by TRF/Cr (AUC 0.540, 95% CI 0.427-0.654) and NGAL/Cr (AUC 0.534, 95% CI 0.420-0.647). RBP/Cr and KIM-1/Cr showed near-chance performance, with AUCs of 0.502 (95% CI 0.389-0.615) and 0.503 (95% CI 0.390-0.615), respectively. Pairwise comparisons of AUCs using the DeLong test revealed no statistically significant differences among the five biomarkers (all $P>0.05$).

Discussion

In the present study, we performed a systematic analysis of the temporal urinary response to high-intensity training in a sub-plateau environment. Urinary NGAL, MCP-1, transferrin, and RBP demonstrated notable, time-related increases, reaching their highest levels 12 hours

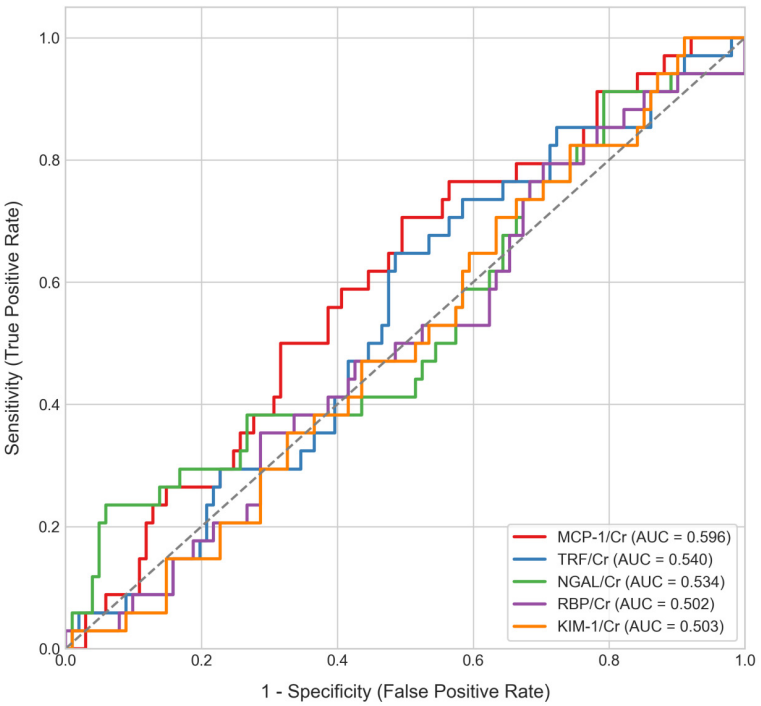


Figure 4. Receiver operating characteristic curves of 12-h urinary biomarkers for predicting acute kidney injury. Abbreviations: MCP-1, monocyte chemoattractant protein-1; TRF, transferrin; NGAL, neutrophil gelatinase-associated lipocalin; RBP, retinol-binding protein; KIM-1, kidney injury molecule-1; Cr, creatinine; AUC, area under the curve.

after training, and then gradually declining to baseline by 48 hours. Furthermore, after adjusting for demographic, hemodynamic, and renal function covariates, NGAL, MCP-1, and transferrin were independently associated with albuminuria (logACR), whereas only MCP-1 exhibited a trend toward an association with eGFR. In contrast, KIM-1, although showing a strong post-exercise surge, was not independently correlated with either functional parameter. These findings indicate that, in the context of combined hypoxia-exercise stress, markers such as NGAL (tubular injury), MCP-1 (inflammation), and transferrin (glomerular permeability) may serve as sensitive, multi-domain indicators of subclinical renal stress, whereas KIM-1 may reflect a more transient or mechanistically distinct aspect of tubular injury.

In the sub-plateau environment, exercise-induced kidney stress is multifaceted, involving hemodynamic changes, ischemia-reperfusion injury, oxidative stress, and inflammatory responses [1, 9]. The combination of hypoxia and intense exercise may compound this stress. According to our findings, the rise in glomerular markers (ACR, transferrin) was followed by a delayed peak (12 hours) in tubular and inflammatory markers, suggesting that initial glomerular hemodynamic stress may precipitate subsequent tubular injury and inflammation. This pattern aligns with the concept of glomerulotubular crosstalk, in which glomerular injury signals propagate to the tubular compartment, thereby exacerbating damage [4].

NGAL is a well-established biomarker of renal tubular damage [5, 14, 15]. After training, NGAL levels increased significantly. Following multivariate adjustment, NGAL showed a significant and independent association with logACR (β = 0.453, P<0.001), consistent with previous findings from marathon and diabetic nephropathy studies [9, 16, 17]. Although NGAL may also originate from extrarenal neutrophils, its persistent correlation with ACR suggests that the renal tubular source is dominant in this setting [18]. NGAL/Cr was not retained in the stepwise regression model for eGFR, suggesting that it may be a more sensitive marker of early structural damage rather than an indicator of overt functional decline.

MCP-1, a chemokine, mediates macrophage infiltration and is highly expressed in glomerular and tubular epithelial tissues during inflammatory conditions [19, 20]. We found that MCP-1/Cr showed the most pronounced increase among all indicators, and had the highest correlation with logACR (r = 0.678, P< 0.001). This marker was not only the strongest independent predictor of logACR (β = 0.707, P<0.001), but also the sole variable retained for eGFR in the stepwise regression (β = 0.162, P = 0.064). This result indicates that the inflammatory pathway reflected by MCP-1 may represent a central mechanism linking the dual stress of hypoxia and exercise to glomerular function impairment and overall functional decline. This finding aligns with evidence that urinary MCP-1 serves as a marker of renal inju-

Table 5. Diagnostic performance of urinary biomarkers at 12 h after training for predicting AKI

Biomarker	AUC (95% CI)	Cutoff	Sensitivity (%)	Specificity (%)	Youden index
MCP-1/Cr	0.596 (0.483-0.710)	2.796	70.6%	50.5%	0.211
TRF/Cr	0.540 (0.427-0.654)	1.974	64.7%	51.5%	0.162
NGAL/Cr	0.534 (0.420-0.647)	0.175	23.5%	94.1%	0.176
RBP/Cr	0.502 (0.389-0.615)	0.029	79.4%	29.7%	0.091
KIM-1/Cr	0.503 (0.390-0.615)	5.415	100.0%	8.9%	0.089

ry progression in diabetic nephropathy [20-23]. Collectively, the prominent performance of MCP-1 underscores the critical role of inflammation in sub-plateau training-related renal injury, which may be further amplified by the hypoxic environment.

Transferrin is a marker of glomerular size-selective permeability. Owing to its higher isoelectric point relative to albumin, it appears in urine earlier than albumin when glomerular damage occurs [24]. In our study, the transferrin-to-creatinine ratio increased approximately fourfold after training, and there was a strong independent correlation with logACR ($\beta = 0.611$, $P< 0.001$). This is consistent with previous observations in diabetic nephropathy, where transferrinuria was related to the severity of glomerular lesions and proteinuria levels [25]. However, unlike MCP-1 and NGAL, the transferrin/Cr ratio showed no independent association with eGFR. This indicates that its increase mainly reflects changes in glomerular permeability rather than a direct relationship with the decline in glomerular filtration function. This dissociation suggests that, in the early, reversible stage of renal functional stress, glomerular barrier dysfunction may occur prior to measurable changes in eGFR.

RBP is a low-molecular-weight protein that is freely filtered by the glomerulus and almost completely reabsorbed by proximal tubular cells, making it a sensitive marker of proximal tubular dysfunction [26]. In our study, RBP/Cr showed a modest but significant post-training increase, peaking at 12 hours, and retained a weak but independent association with logACR ($\beta = 0.309$, $P<0.001$) after full adjustment. This finding is consistent with previous studies in which urinary RBP increased with proteinuria and reflected impaired tubular reabsorption [26, 27]. The relatively modest elevation of RBP compared with NGAL and MCP-1 in our cohort suggests that, although proximal tubular dysfunction is present, it is not the dominant feature of renal stress in this setting.

KIM-1 is a transmembrane protein expressed in proximal tubular cells following ischemic or toxic injury, and its urinary excretion is considered a highly sensitive marker of structural tubular damage [28, 29]. In our study, KIM-1/Cr demonstrated the largest relative increase of all markers at 12 hours post-training. However, despite this dramatic surge, KIM-1/Cr showed no significant correlation with either eGFR or logACR, and was excluded from all regression models. Furthermore, its ROC analysis revealed 100% sensitivity but only 8.9% specificity for KDIGO-defined AKI, underscoring a profound dissociation between structural stress and functional decline. This apparent paradox can be explained by several interrelated mechanisms. First, KIM-1 is not merely a passive damage marker; it functions as a phosphatidylserine receptor that recognizes and internalizes apoptotic cells and oxidized lipids, thereby actively promoting tubular epithelial repair and restoration of tissue integrity [30]. Thus, in a reversible, exercise-induced stress setting, its robust upregulation may reflect an adaptive regenerative program rather than irreversible injury, which would uncouple its elevation from contemporaneous declines in glomerular filtration. Second, urinary KIM-1 arises from metalloproteinase-mediated cleavage of the extracellular domain, a shedding process that can be stimulated by oxidative stress and inflammatory cytokines independently of changes in GFR [31]. Consequently, massive KIM-1 excretion can occur even when nephron filtration capacity remains intact, explaining the lack of correlation with eGFR at a single time point. Third, this phenomenon is particularly pronounced in the setting of strenuous physical exertion. Wołyniec et al. reported a pronounced increase in urinary KIM-1 without a corresponding rise in NGAL after a 100-km ultramarathon, concluding that KIM-1 primarily signals tubular

structural stress rather than functional glomerular impairment [32]. Similarly, Mansour et al. found that urinary KIM-1 and IL-18 increased significantly after marathon running while serum creatinine remained unchanged, again illustrating that tubular injury markers can be dissociated from functional AKI in exercising populations [16]. Fourth, the limited predictive ability of KIM-1 for creatinine-based AKI has been consistently demonstrated across broader clinical contexts. In a large meta-analysis, Ho et al. reported a pooled AUC of only 0.72 for KIM-1 in predicting AKI, reflecting high sensitivity but poor specificity [33]. Endre et al. further showed that the discriminative performance of urinary KIM-1 and NGAL was heavily influenced by baseline renal function and injury duration, and cautioned against their use as standalone diagnostic tools for functional AKI [34]. Collectively, these data indicate that in young, healthy individuals subjected to a single bout of high-intensity exercise under hypobaric hypoxia, a pronounced KIM-1 surge represents an early, largely reversible tubular structural response that is mechanistically and temporally uncoupled from reductions in GFR. Therefore, in this specific population, KIM-1 may best serve not as a predictor of creatinine-defined AKI, but as a sensitive marker of exposure to tubular stress, providing complementary information to functional indices.

The observation that most urinary biomarkers reached their highest levels at 12 hours after exercise identifies a potentially informative time point for monitoring acute renal responses in individuals performing strenuous running exercise. The independent associations of NGAL, MCP-1, and transferrin with albuminuria suggest their utility as reliable adjuncts to conventional markers for early detection of renal stress. Furthermore, the discordant behavior of KIM-1 highlights the importance of employing a panel of biomarkers that capture distinct pathophysiological domains injury (NGAL), inflammation (MCP-1), and glomerular permeability (transferrin), rather than relying on a single marker. Direct comparison with two landmark marathon studies highlights the distinct temporal profile of renal biomarker responses in our cohort. Mansour et al. reported that after the Hartford Marathon, urinary KIM-1, NGAL, and MCP-1 peaked immediately post-race and returned to baseline within 24 h [16]. Atkins et al. observed that after the Boston Marathon, urinary NGAL remained significantly elevated at 24 h, a sustained response attributed to the course's downhill eccentric loading, warmer temperatures, and faster finishing times [9]. In the present study, most biomarkers peaked at 12 h and normalized by 48 h, occupying an intermediate position, more prolonged than the Hartford pattern yet more self-limited than the Boston pattern. This temporal shift likely reflects the superimposed hypobaric hypoxia unique to sub-plateau training. These comparisons underscore that the magnitude and time course of exercise-induced renal stress depend heavily on the specific combination of intensity, environment, altitude, and mechanical load. Future studies should investigate whether these biomarkers can identify individuals at risk for recurrent injury or long-term sequelae, and whether targeted interventions can modulate these responses.

Limitations

This study has certain limitations. First, the modest number of AKI events (n = 34, 25.2%) limits the statistical power of the ROC analysis; therefore, the low AUCs should be interpreted cautiously. Furthermore, in exercise settings, tubular injury biomarkers often rise without a concomitant increase in Scr, which suggests that their primary value here lies in detecting subclinical renal stress rather than predicting creatinine-based AKI. Second, we did not measure core body temperature, hydration status (e.g., plasma osmolality), or markers of systemic inflammation and oxidative stress, which could help elucidate the mechanistic pathways linking exercise to renal injury. Third, our study population consisted exclusively of young, healthy male participants; thus, the results may not be generalizable to women, older individuals, or those with pre-existing comorbidities. Fourth, the 48 h follow-up period, although sufficient to capture the acute response, may not detect more prolonged or delayed effects of the dual stressor. Finally, the observational design precludes causal inferences regarding the relationship between biomarker elevations and long-term renal outcomes.

Conclusions

Following sub-plateau high-intensity training, urinary NGAL, MCP-1, transferrin, and RBP

exhibited significant transient elevations. NGAL, MCP-1, and transferrin were independently associated with albuminuria, supporting their utility as multi-domain markers of renal stress. In contrast, KIM-1, despite a marked post-exercise surge, did not correlate with functional parameters, suggesting a distinct role in reversible subclinical injury. These findings support the use of a panel comprising NGAL and MCP-1 for early detection of renal stress in populations exposed to combined hypoxic and exertional stress, which warrants validation in larger, diverse cohorts and further investigation into long-term implications.

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Disclosure of conflict of interest

None.

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