Bilirubin as an Adaptive Oxidative Stress Biomarker in Judo Athletes: A Pilot Study Using Non-invasive Biological Matrices

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Abstract: *This pilot study evaluates bilirubin as a potential adaptive biomarker of psychophysical and oxidative stress in judo athletes, moving beyond its traditional role in liver function assessment. Bilirubin and its conjugate were quantified in nine athletes before and after training and competition using the high throughput fluorometric 'HUG' assay. Results were compared with cortisol, amylase and malondialdehyde levels across blood, urine and saliva samples.*

Intense exercise induced significant post-activity increases in bilirubin which correlated positively with established oxidative and psychophysical stress markers. Notably, urine emerged as the most sensitive and effective matrix for non-invasive monitoring of bilirubin fluctuations. The findings suggest that bilirubin could serve as a potential biomarker of antioxidant response and recovery. Incorporating this biomarker in non-invasive monitoring protocols may contribute to the enhancing of practical tools for optimising workload and reducing the risk of overtraining in elite sport.

Keywords: *Biomarkers, bilirubin; cortisol, amylas;, oxidative stress, non-invasive monitoring*

An understanding of the impact of exercise-induced psychophysiological stress has received considerable attention in recent years, regarding performance and fatigue. Most studies conducted over the past two decades have consistently reported an increase in oxidative stress following acute exercise, whether aerobic or anaerobic, supporting the concept that exercise induces oxidative stress regardless of the type or intensity of activity (Fisher-Wellman & Bloomer, 2009; Kawamura & Muraoka, 2018; Squillacioti et al., 2021). Judo, in particular, is an acyclic sport for which performance depends on a combination of different physical abilities. It is a sport with high intensity and intermittent actions. Therefore, both the aerobic and anaerobic metabolic systems are alternately stimulated (Franchini et al., 2011; Gandouzi et al., 2023).

Physical activity is closely associated with oxidative stress, a condition defined by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses (Power et al., 2020). During intense or prolonged exercise, the elevated oxygen consumption in muscles promotes the generation of ROS and reactive nitrogen species which can damage lipids, proteins and DNA, contributing to muscle fatigue, inflammation, and, in severe cases, an increased risk of overtraining (Finaud et al., 2006) and injuries (Lewis et al., 2020; Wang, 2021; Lui et al., 2022).

However, moderate levels of ROS play a positive role: they activate cellular signaling pathways, promote muscle adaptation and enhance endogenous antioxidant systems such as superoxide dismutase, catalase and glutathione peroxidase (Power et al., 2020). This phenomenon, known as hormesis, explains why regular exercise reduces the risk of chronic diseases and premature ageing (Meng & Su, 2024). Balance is therefore crucial: calibrated workouts and a diet rich in natural antioxidants improve the ability to counteract oxidative stress, while excessive and untargeted supplementation can hinder beneficial adaptations. An intriguing idea is to elevate endogenous antioxidants through physical activity, creating a virtuous cycle in which controlled stress produces a beneficial oxidative status that amplifies health benefits.

The use of biomarkers has become an essential monitoring tool in the field of sport and exercise medicine. Among these, in addition to being a good biomarker of oxidative stress, bilirubin is an excellent candidate as a powerful antioxidant. Bilirubin is recognised as a potent endogenous antioxidant, more effective than vitamin E at neutralising reactive oxygen species (Woronyczová et al., 2022). These properties suggest that moderately elevated bilirubin concentrations may provide adaptive and protective advantages in response to oxidative and inflammatory stress, reducing cardiovascular risk (Zuo et al., 2022) and lowering the incidence of metabolic syndrome (Nikouei, 2024).

Athletes, especially elite athletes, tend to have higher bilirubin levels than sedentary individuals. This appears to be a protective adaptation to the increased oxidative and inflammatory stress caused by intense exercise. Current

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evidence suggests that these elevations may generally reflect physiological adaptation rather than pathology (Witek et al., 2017). These authors reported that 18 – 20% of athletes display bilirubin levels above the reference range established for the general population, in the absence of clinical abnormalities, and proposed athlete-specific limits with an upper threshold of approximately 29 µmol/L. Consequently, bilirubin should be interpreted not only as a marker of liver damage but also as a potentially beneficial factor for health and athletic performance (Flack et al., 2023).

The transition from understanding bilirubin's biological role to its practical application in sports science lies in its sensitivity to training volume and intensity. Unlike static clinical markers, bilirubin serves as a dynamic indicator of the athlete's current redox status (Di Gioia et al., 2023). By tracking its fluctuations, practitioners can distinguish between beneficial physiological adaptation (hormesis) and excessive oxidative debt that may lead to maladaptation. Therefore, incorporating bilirubin into routine screening could extend beyond traditional liver health assessment and may provide a non-traditional metric to gauge recovery and quantify the metabolic cost of high-intensity effort, such as those encountered in judo.

Monitoring athletic performance during training and competition requires analytical methods that are fast, simple and inexpensive, along with non-invasive sampling techniques which are capable of indicating any psychological or physical imbalance at an early stage to prevent failure or injury.

RESEARCH AIMS AND HYPOTHESES

Based on the current gap in literature regarding multi-matrix stress assessment in combat sports such as judo, the primary objective of this pilot study is to evaluate the feasibility of using bilirubin as a potential adaptive biomarker for athlete monitoring by comparing its levels with standard psychophysiological stress markers across different biological matrices (urine, saliva and blood). The specific objectives were the assessment of the acute response of bile pigments and stress parameters before and after exposure to standardised training and competitive judo events, identifying individual patterns of oxidative stress.

We hypothesise that (1) bilirubin levels will show significant acute fluctuations in response to judo-specific loads, reflecting its consumption as a primary antioxidant, and (2) these variations will correlate with changes in established stress biomarkers, thereby validating bilirubin as a reliable and accessible indicator of an athlete's psychophysiological state. The final hypothesis is that higher bilirubin levels may contribute to metabolic and performance-related benefits, such as increased endurance, vascular protection and improved fat oxidation.

METHODS

The experimental design presents this study as an exploratory and feasibility study with an embedded case observation on the use of bilirubin as an additional marker among indicators of athletes' oxidative status.

Subjects and sampling protocol

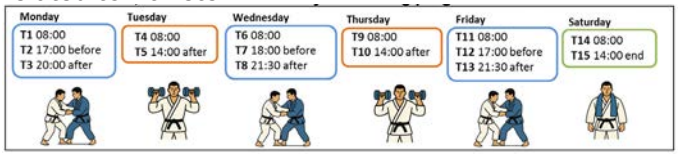
Nine medium-level *judoka* (four male, five female) from the Friuli Venezia Giulia region, Italy, were recruited. The study was submitted to and approved by the ethics committee of the University of Trieste. All participants confirmed they were in good health, were non-smokers and were not taking any supplemental vitamins or antioxidants, following an established protocol (Sist & Urbani, 2022). One of these athletes was then selected for closer observation during a one-week judo session to determine parameter values across the three different biological fluids. Biometric parameters for all participants are reported in Table 1.

Table 1. Subjects physical characteristics.

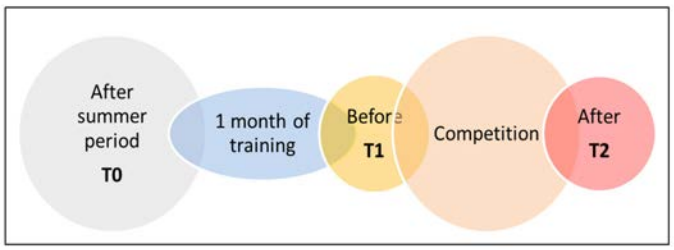
	#	Age (years)	Judo practice (year)	Training (h/week)	Hight (m)	Weight (kg)	Body Mass Index (kg/m^2)
Male	1	22	18	8	1,90	128	35.5
	2	18	11	5	180	90	27.8
	3	17	12	10	164	63	23.7
	4	16	7	8	177	90	28.7
Female	5	27	22	8	160	60	23.4
	6	18	13	8	168	65	23
	7	16	7	5	157	55	22.3
	8	14	8	4	159	53	21
	9	14	8	5	153	63	26.9

Blood, saliva and urine samples for athlete 1 were collected as shown in Scheme 1, while urine and saliva samples from the other athletes were collected as shown in Scheme 2.

Scheme 1. Blood, saliva and urine weekly programme related to Athlete 1.



Scheme 2. Monitoring programme including a competition event.



Samples were self-collected, non-invasively, by the athlete at home or in the gym. After receiving thorough instructions, saliva was collected, without stimulation, using a sterile Salivette (Sarstedt®) and urine was collected in a sterile 50 ml Falcon tube. These samples were trans-

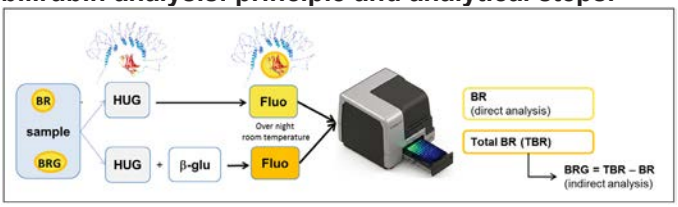
ported promptly to the laboratory and frozen at -80°C for analysis (Sist et al., 2022). Capillary blood was obtained using a finger-prick device. The resulting drop was collected in a sterile vial and transferred immediately by an operator using an automatic pipette.

HUG fluorometric assay

The quantification of bilirubin (BR) and its congener bilirubin glucuronide (BRG) employed a high-throughput fluorometric method (Scheme 3) based on the selective high-affinity binding of bilirubin to the HELP-UnaG (HUG) fusion protein (Sist & Urbani, 2022; Sist et al., 2023; Sist et al., 2025). The assay can be enhanced for the measurement of conjugated bilirubin (BRG) by adding the enzyme beta-glucuronidase to the reaction mixture (Pelizzo et al., 2023).

Small sample volume (5 µL of blood, 200 µL of saliva, or 100 µL of urine) was added to HUG solution with or without β-glucuronidase enzyme (β-glu).

Scheme 3. HUG method of bilirubin and conjugated bilirubin analysis: principle and analytical steps.



Biochemical parameters

Creatinine (CRE) was determined using the Jaffe method (1886). Cortisol was extracted from saliva and urine with dichloromethane and analysed via HPLC-UV based on a method by Pihut et al. (2015) with modifications. Malondialdehyde (MDA) was measured in all matrices by HPLC with fluorescence detection after derivatisation with thiobarbituric acid, following Agarwal & Chase (2002). Amylase activity in saliva and urine was quantified using a commercial assay kit (Sigma-Aldrich).

Statistical methods

Using R and ggplot2, a Kendall correlation matrix was generated to assess statistical associations between variables. Kendall's τ coefficient was used because it is non-parametric and suitable for non-normal distributions and provides accurate correlations with limited data points. Given the exploratory nature of this study, no correction for multiple testing was applied. Results should be interpreted as preliminary and require replication in a confirmatory study.

RESULTS AND DISCUSSION

Bilirubin is a molecule with multiple functions, such as antioxidant, anti-inflammatory and regulatory effects (Schwertner & Witek, 2008; Vitek & Ostrow, 2009; Hammouda et al., 2012; Creeden et al. 2021) Moreover, in high-intensity training, bilirubin acts as a sophisticated signalling molecule that helps the body adapt to physical stress (Witek et al., 2017). Assessing baseline bilirubin levels in saliva and their changes after stressful situations is an innovative measure that is not currently used, as the concentrations are very low and cannot be detected by conventional analytical methods. In this work, we present preliminary results for biomarkers measured in different matrices (urine, saliva and blood) to assess the optimal analytical protocol and the matrix effect.

Bilirubin recovery test

A spike-and-recovery test was conducted, as recommended for previously untested sample types, by adding known amounts of BR to known volumes of a single pool of saliva and urine specimens, to assess potential interference from the analytical matrices. To experimentally determine the expected BR recoveries, identical spiking was performed on the solvent of the standard solution used for assay calibration, consisting of 4 g/L bovine serum albumin in phosphate-buffered saline at pH 7.4. As shown in Table 1, no significant difference in BR concentration was found between saliva, urine and the BSA solution, referred to as the observed and expected values, respectively. The low standard deviation indicates good reproducibility of the test. This data indicates that no matrix effect is present in the assay.

Table 1. Recovery of BR in blood, urine and saliva under spiking conditions.

Sample (n)	Spike Level, nM	Expected BSA	Observed	Recovery %	*p*-value
Blood (n=2)	Low	9.4 (±0.5)	9.5 (±1.4)	101	*0.309*
	Med	26.0 (±2.2)	25.8 (±3.8)	99	*0.735*
	High	46.2 (±1.6)	46.6 (±1.9)	101	*0.046*
Urine (n=2)	Low	10.3 (±0.1)	11.6 (±0.4)	112	*0.434*
	Med	24.3 (±1.0)	24.6 (±0.1)	101	*0.255*
	High	47.7 (±3.4)	48.5 (±0.6)	101	*0.192*
Saliva (n=2)	Low	10.9 (±0.1)	10.8 (±1.1)	98	*0.839*
	Med	25.7 (±1.1)	24.6 (±1.1)	96	*0.100*
	High	50.2(±0.4)	49.4 (±0.6)	98	*0.161*

n represents the number of independent replicates performed on pooled biological fluid. Results were analyzed by 2-way ANOVA test using standard significance level α = 0.05.

Quantification of BR and BRG

Figure1 shows that BR and BRG values in the blood of athlete 1 are in the same µM range and remain within physiological ranges of 11 – 21 µM and 0 – 0.7 µM, respectively. In addition, excess BR resulting from physical activity appears to be rapidly eliminated as BRG, particularly through urine and also through bile, to keep levels below toxic limits.

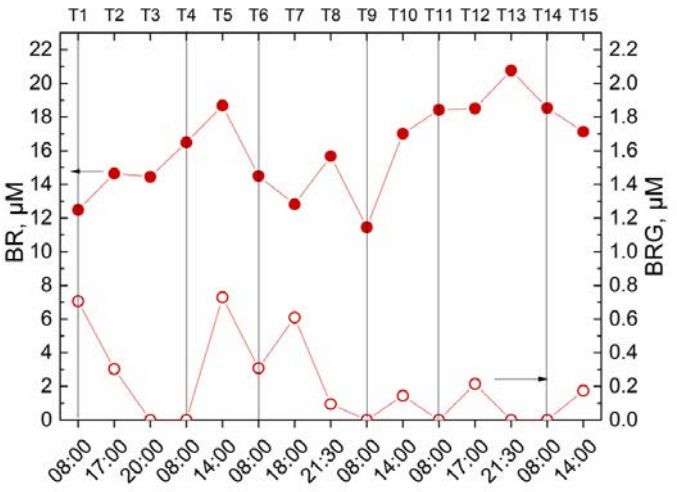


Figure 1. Bilirubin (BR) and conjugated bilirubin (BRG) concentrations in blood samples during the training week of athlete 1.

In urine, BR and BRG values are in the same nmol/g creatinine range, as shown in Figure 2A. BR and BRG in morning urine samples at 8:00 (T1, T4, T6, T9, T11, and T14) have average values of 3.4 ± 0.9 nmol/gCRE and 2.8 ± 0.7 nmol/g, respectively, as reported in Figure 2A. In contrast, these parameters show a marked increase in concentration after a training session, rising up to the 11.5 nmol/g CRE for BR and up to 6.9 nmol/g CRE for BRG (peeks in Figure 2A).

In saliva samples (Figure 2B), BR values were always higher than BRG, while in urine BRG values tended to remain in the same range as BR, consistent with BR metabolic clearance. The concentrations of BR and BRG in saliva were, on average, lower than those detected in urine without creatinine correction (data not shown), where the values were also more sensitive to variations caused by physical activity.

The peaks in Figures 2A and 2B indicate the highest values of the variables BR and BRG, occurring half an hour after training in both matrices.

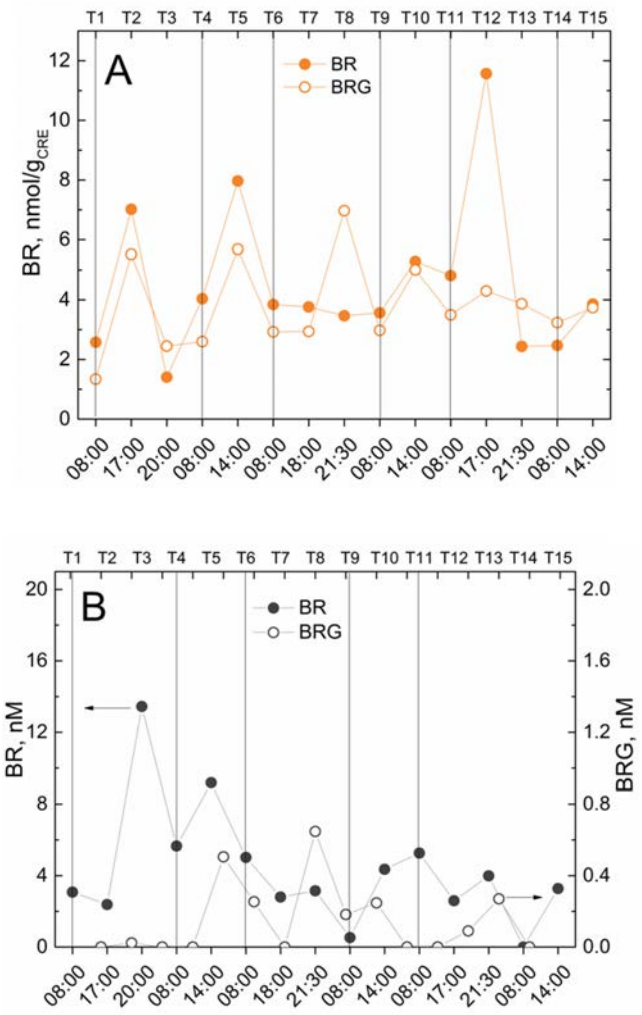


Figure 2. BR and BRG in (A) urine and (B) saliva during the training week.

Quantification of other stress biomarkers

In the present study, the biomarkers of oxidative stress — namely MDA, amylase, and cortisol — were analysed before and after training. In the previous paper (Sist & Urbani, 2022) the main findings were that aerobic and anaerobic judo training may cause a general increase in oxidative stress when the antioxidant system is inefficient in responding to free radical production.

Creatinine was also measured during the training week. While the normal reference range in adults is 0.7 – 1.4 g/L, levels in athletes are usually higher, depending on training load; therefore, specific reference values for athletes have not yet been well defined (Palacios et al., 2015). In cases of elevated creatinine concentration (>1.4 g/L), caution is advised, as high values do not necessarily indicate kidney damage. In this work, an average value of 2.9 ± 0.9 g/L of creatinine was obtained going from 1.4 g/L up to 5.2 g/L with higher values after training (3.0 - 5.2 g/L).

Salivary, urinary and blood MDA concentrations are reported in Figure 3. MDA values in urine appeared more sensitive to physical activity than those measured in blood or saliva. Therefore, in our study, urine specimens appeared to be more sensitive in the MDA monitoring assessment.

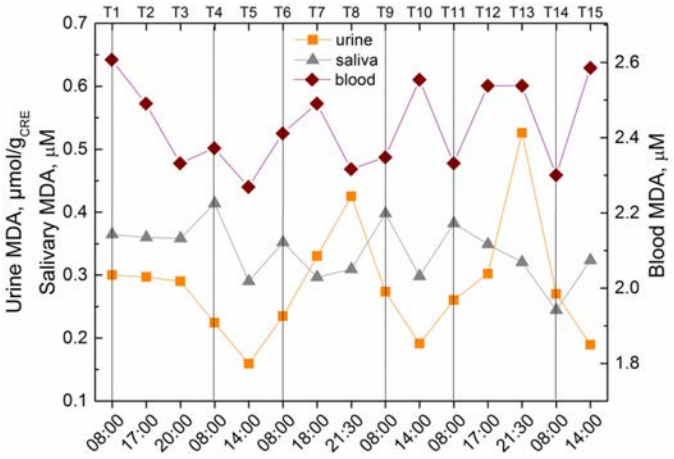


Figure 3. MDA concentrations in urine, saliva and blood during the training week.

Cortisol (CORT) and α-amylase (AA) are distinct indicators of the stress response. Their interaction is essential for understanding the effects of psychophysical stress on athletes and depends on several factors, such as training stress, intensity and the psychological response to the load. The salivary and urinary cortisol concentrations after different physical activities are reported in Figure 4A. Compared to the measurements at pre-training judo events (T1, T6, and T11), both urinary and salivary cortisol increased significantly at post-training phases T3, T7 and T13, with wide variations observed for urinary cortisol (Figure 4A). The results highlighted that high- and middle-intensity training increases the body's cortisol and α-amylase levels differently in both saliva and urine. We observed significant differences in the rates of change in cortisol concentrations resulting from early morning exercise programmes of varying intensities on different days. More precisely, greater variability is observed in urinary cortisol (Figure 4A), while conversely, greater variability is observed in salivary α-amylase (S-AA) (Figure 4B) with rapid response with marked peak values at the end of the physical exercise.

Physical exercise of moderate and high intensity caused an increase in heart rate above 100 bpm, largely due to increased sympathetic activity, which could explain the rise in the production and appearance of α-amylase in saliva after training. Much more than cortisol, which tends to remain elevated during and after peak stress, α-amylase showed a more rapid pattern, peaking immediately after a stressful event and then returning to the baseline quickly (Figure 4B). This makes α-amylase a useful indicator for detecting immediate changes in stress activation, whereas cortisol provides a more comprehensive and prolonged view of the hormonal response.

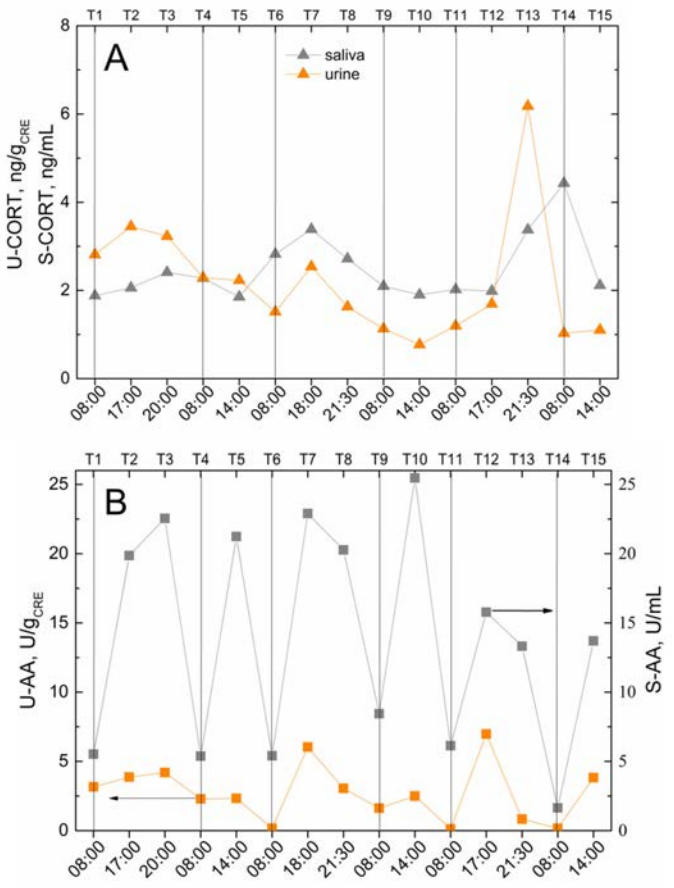


Figure 4. (A) Salivary and urinary cortisol concentrations; (B) salivary and urinary amylase concentrations during training week.

The main findings of this study confirmed that S-AA and S-CORT may provide valuable information for coaches regarding athletes' responses to training and competition. Increases in basal cortisol concentrations after periods of intensified training have already been reported in the literature and this increase markedly depends on the training status of the athlete.

Effect of competition on bilirubin level and on oxidative stress parameters

In high-intensity sports such as judo, which are characterised by explosive efforts and intense emotional pressure, competitions can generate significant physiological and psychological responses, including increased oxidative stress and psychological stress in athletes. From a psychological perspective, anticipation of competition triggers activation of the hypothalamic-pituitary-adrenal axis and increases salivary cortisol (Filaire et al., 2007), especially in the moments before competition (Salvador et al., 2003). Amylase and cortisol are complementary biomarkers of stress (Ciaccioni et al., 2024; Kivlighan & Granger, 2006; Salvador et al., 2003) and the interaction between these two biomarkers is complex, depending on several factors, including the duration and intensity of stress, the athlete's training level, and their psychological response to the load. These psychophysiological parameters (S-AA and

S-CORT), in addition to oxidative stress parameters like BR, were measured in eight athletes (2–9) during three periods (T0, T1, and T2), as schematically shown in Scheme 2.

Bilirubin is a molecule with multiple functions and has important antioxidant, anti-inflammatory and regulatory effects. Assessing baseline bilirubin levels in saliva and their changes after stressful situations could be considered an innovative measure that is not used currently, as the concentrations are very low and cannot be detected by conventional analytical methods.

Total BR (TBR as the sum of BR and BRG values) in urine was measured and the results are presented in Figure 5. Almost all the athletes showed a marked increase in TBR just before (T1) and after competition (T2) compared with T0. The number on the top of the bars in Figure 5 indicates the S-CORT concentration (ng/mL). Higher cortisol values correspond to higher TBR values.

Salivary amylase, for three male athletes (2, 3 and 4), was measured and assessed in comparison to urinary TBR and S-CORT, presented in Figure 5. S-AA (Table 2) showed a significant increase from the rest period (T0) before the competition to 1 hour before the competition (T1); then showed a trend towards a decrease after the event (Table 2), indicating higher levels of both anxiety and physical stress.

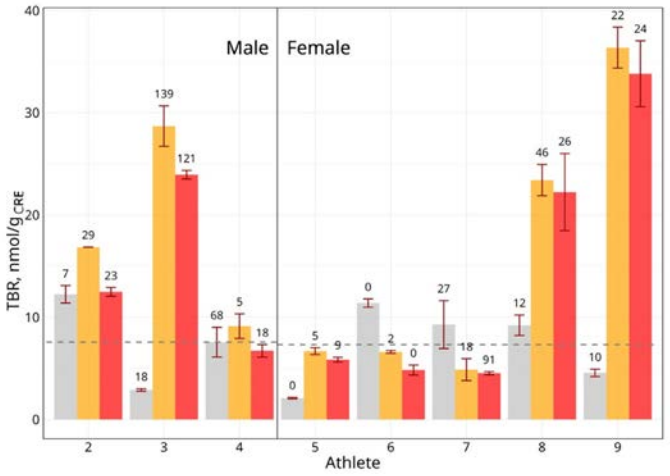


Figure 5. Total BR in urine (bars) and S-CORT values (numbers on the bars as ng/mL) of athletes before and after judo competition. (Grey bar = T0, yellow bar = T1, red bar = T2; dashed line indicates the average basal value T0 = 7.3 ± 3.7 nmol/gCRE and 7.6± 4.1 nmol/gCRE for female and male, respectively).

Meanwhile, S-CORT showed a marked increase from the rest period T0 to T1 before the competition and remained at higher levels. This increase is related to the psychophysical condition, including anxiety, which may remain higher even after the physical competition has ended. In these three subjects, the results for the three parameters differ markedly according to their varying levels of fitness and psychophysical maturity, as reported in Figure 5 and Table 2.

Table 2. Salivary amylase for athletes 2, 3 and 4 before and after judo competition

Sample	Athlete 2	Athlete 3	Athlete 4
T0	5.53	8.09	13.73
T1	9.55	9.23	28.21
T2	4.88	10.63	23.48

The present findings provide valuable information on the differences in temporary changes in stress-related parameters during real competition and training and also indicate the chronic changes that occur after intensive training in well-trained athletes. These results suggest that there may be additional correlations between psychological and physiological factors that could lead to reduced athletic performance in both competition and training. In conclusion, elevated S-AA and S-CORT levels from immediately before the competition until after its end may be factors in reduced performance.

Correlation analysis between all the parameters

All results obtained for the nine athletes were processed statistically and the Kendall correlations are presented in the matrix in Figure 6. The sample size is small and the significance obtained may be affected by this; therefore, this analysis should be considered preliminary. However, some promising correlations are highlighted to support the use of BR as an additional marker in the analysis of the oxidative status of a judo athlete. There was a positive correlation (τ= 0.283, p = 0.028) between total urinary BR (U-TBR) and total blood BR (B-TBR), suggesting that urine might reflect blood BR levels, although further validation is required. There is also a weak correlation (τ = 0.383, p = 0.059) between salivary and urinary BRG. However, as shown in Figure 2, urine values are more sensitive to metabolic changes related to sports activity.

Intense exercise can increase bilirubin levels in blood and urine temporarily, especially if muscle damage is involved (e.g., through eccentric exercise) or if there are changes in liver metabolism. However, a fairly strong correlation between U-TBR and B-TBR (blood) (= 0.283, p = 0.028) suggests that bilirubin levels may indicate how the body manages detoxification processes in both the liver and kidneys. S-CORT correlated well with urinary amylase (U-AA) (= -0.534, p<0.001), U-BR (= 0.243, p = 0.029), and U-MDA (= 0.335, p = 0.003), suggesting good correlations between psychological and physiological parameters which may lead to reduced athletic performance during both competition and training.

Cortisol is a stress hormone that rises with intense physical activity and psychological stress. If exercise is sufficiently

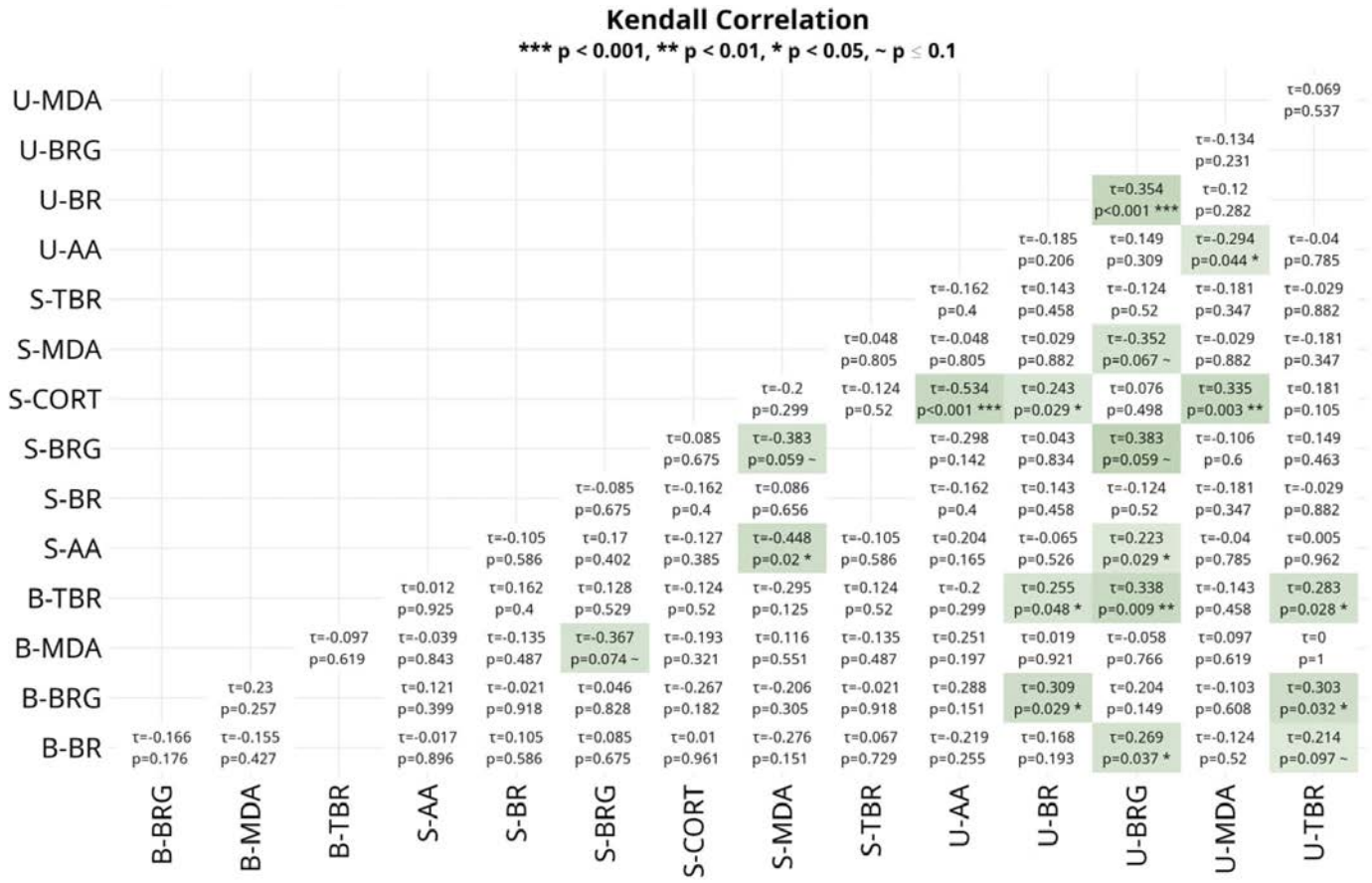


Figure 6. Kendall correlation matrix between all the parameters considered

intense to elevate cortisol, it is likely that oxidative damage and MDA production will also increase. The negative strong correlation between U-AA and S-CORT reflect a physiological response to recovery where cortisol decreases after exercise as the body enters the recovery phase. On the contrary, the increase in post-exercise cortisol may also indicate a high load on the nervous system and metabolism. If cortisol remains high, it may indicate excessive stress or insufficient recovery capacity. Moreover, increased U-AA may indicate heightened physiological stress associated with the depletion of energy resources, such as during post-exercise recovery, when the body is working to restore energy reserves. The response of biomarkers such as S-AA and U-BRG (= 0.223, p = 0.029) may indicate how the body adapts to and manages exercise. If these biomarkers are chronically elevated, it may be necessary to review the intensity and frequency of exercise sessions.

Bilirubin levels in urine, saliva and blood are generally positively correlated, which is consistent with physiological expectations. However, variables such as amylase, cortisol and malondialdehyde show weaker correlations, although there are some significant associations, such as between amylase and cortisol. Correlations with cortisol are noteworthy as they suggest possible interactions between psychophysical stress (indicated by cortisol) and bilirubin-related metabolism or detoxification processes.

Serum bilirubin levels are influenced by physiological factors described in the literature. Fasting increases unconjugated bilirubin due to reduced hepatic enzyme activity and increased enterohepatic circulation (Barrett, 1971; Fevery, 2008). The variation in bilirubin during fasting can be significantly greater in males than in females (Griffin et al., 2014). Hydration status can also cause an apparent increase in values due to haemoconcentration from reduced plasma volume (Karila et al., 2008). In addition, bilirubin concentration follows a distinct circadian rhythm, with higher inter-individual variation during the day (Larson et al., 2009), and may depend on the menstrual cycle and hormonal fluctuations (Yamaguchi et al., 1975). Considering all these factors is essential for proper interpretation of results. However, an assessment of potentially confounding factors is aided by the dietary, training and medical monitoring that elite athletes undergo.

By combining biomarkers of oxidative stress (MDA), endocrine stress (cortisol) and sympathetic response (salivary amylase) with antioxidant markers (bilirubin) in accessible matrices such as saliva and urine, it is possible to create a personalised biochemical profile to assess internal load, monitor recovery, prevent overtraining, and optimise performance.

LIMITATIONS

Despite promising results, this study has several limitations that should be considered when interpreting the results. The sample size was small (N = 9), which may limit the statistical power and generalisability of the findings. In addition, the cohort lacked sufficient diversity in

age groups, training modalities and sport disciplines (e.g., endurance versus power sports). Future research should use a larger, more heterogeneous sample and include additional psychological assessments to further clarify the relationship between bilirubin and psychophysical stress.

CONCLUSIONS

Our preliminary findings suggest that bilirubin and its derivative, bilirubin glucuronide, may act as more than simple by-products of liver metabolism, potentially serving as biomarkers in the response to oxidative stress induced by intense sporting activity, such as judo. Bilirubin, in particular, suggests notable antioxidant and anti-inflammatory properties in neutralising reactive oxygen species. Analysis of different biological matrices (blood, urine, saliva) has shown that bilirubin levels increase significantly in response to training sessions or competitions, in parallel with other biomarkers of psychophysical stress such as cortisol, amylase and malondialdehyde. In this pilot study, urine emerged as a potentially sensitive matrix for monitoring bilirubin levels, showing an increasing trend after exercise and before and after competition, probably as a protective mechanism.

The results suggest that bilirubin may be regarded not only as an indicator of liver damage but also as a marker of the body's adaptation to increased oxidative and inflammatory stress. In this context, well-trained athletes physiologically exhibit higher bilirubin levels, indicating a possible protective advantage. These findings suggest the potential for the use of bilirubin as an accessible, non-invasive and informative biomarker for monitoring health status, recovery and the risk of overtraining in athletes, supporting a more personalised and preventative approach in sports medicine.

The authors thank the FVG judo athletes, their coaches and clubs for participating in this study. The experiments comply with the current laws of the country in which they were conducted.

REFERENCES

Agarwal, R., & Chase, S. D. (2002). Rapid, fluorimetric-liquid chromatographic determination of malondialdehyde in biological samples. Journal of chromatography. B, *Analytical technologies in the biomedical and life sciences, 775*(1), 121–126. https://doi.org/10.1016/s1570-0232(02)00273-8

Barrett, P. V. (1971). Hyperbilirubinemia of fasting. *JAMA, 217*(10), 1349-1353.

Boon, A. C., Sayakhot, P., Bulmer, A. C., Coombes, J. S., Fassett, R. G., & Wagner, K. H. (2014). Circadian rhythms of molecular clock gene expression and function in human adipose tissue. *Molecular Genetics and Metabolism, 111*(3), 337-343.

Ciaccioni, S., Martusciello, F., Di Credico, A., Guidotti, F., Conte, D., Palumbo, F., Capranica, L., & Di Baldassarre, A. (2024). Stress-Related Hormonal and Psychological Changes to Simulated and Official Judo Black Belt Examination in Older Tori and Adult Uke: An Exploratory Observational Study. *Sports, 12*(11), 310. https://doi.org/10.3390/sports12110310

Creeden, J. F., Gordon, D. M., Stec, D. E., & Hinds, T. D., Jr (2021). Bilirubin as a metabolic hormone: the physiological relevance of low levels. *American journal of physiology. Endocrinology and metabolism, 320*(2), E191–E207. https://doi.org/10.1152/ajpendo.00405.2020

Di Gioia, G., Crispino, S. P., Monosilio, S., Maestrini, V., Nenna, A., Segreti, A., Squeo, M. R., Lemme, E., Ussia, G. P., Grigioni, F., & Pelliccia, A. (2023). Cardiovascular and metabolic effects of hyperbilirubinemia in a cohort of Italian Olympic athletes. *Scandinavian journal of medicine & science in sports, 33*(12), 2534–2547. https://doi.org/10.1111/sms.14481

Felsher, B. F., Rickard, D., & Redeker, A. G. (1970). Hepatic bilirubin glucuronidation in Gilbert's syndrome. The Journal of Clinical Investigation, 49(12), 2424-2435

Fevery, J. (2008). Bilirubin in clinical practice: a review. *Liver International, 28*(5), 592-605. doi:10.1111/j.1478-3231.2008.01716.x

Filaire, E., Rouveix, M., Alix, D., & Le Scanff, C. (2007). Motivation, stress, anxiety, and cortisol responses in elite paragliders. *Perceptual and Motor Skills, 104*(3_suppl), 1271-1281.

Finaud, J., Lac, G., & Filaire, E. (2006). Oxidative stress : relationship with exercise and training. *Sports medicine (Auckland, N.Z.), 36*(4), 327–358. https://doi.org/10.2165/00007256-200636040-00004

Fisher-Wellman, K., & Bloomer, R. J. (2009). Acute exercise and oxidative stress: a 30 year history. *Dynamic medicine : DM, 8*, (1). https://doi.org/10.1186/1476-5918-8-1

Flack KD, Vítek L, Fry CS, Stec DE, Hinds TD Jr. (2023) Cutting edge concepts: Does bilirubin enhance exercise performance? *Front Sports Act Living, 11* (4):1040687. doi: 10.3389/fspor.2022.1040687. PMID: 36713945; PMCID: PMC9874874

Franchini, E., Del Vecchio, F. B., Matsushigue, K. A., & Artioli, G. G. (2011). Physiological profiles of elite Judo athletes. *Sports medicine (Auckland, N.Z.), 41*(2), 147–166. https://doi.org/10.2165/11538580-000000000-00000

Gandouzi, I., Fekih, S., Selmi, O., Chalghaf, N., Turki, M., Ayedi, F., Guelmami, N., Azaiez, F., Souissi, N., Marsigliante, S., & Muscella, A. (2023). Oxidative status alteration during aerobic-dominant mixed and anaerobic-dominant mixed effort in judokas. *Heliyon, 9*(10), e20442. https://doi.org/10.1016/j.heliyon.2023.e20442

Griffin, P. M., Elliott, S. L., & Manton, K. J. (2014). Fasting increases serum bilirubin levels in clinically normal, healthy males but not females: a retrospective study from phase I clinical trial participants. *Journal of clinical pathology, 67*(6), 529–534. https://doi.org/10.1136/jclinpath-2013-202155

Hammouda, O., Chtourou, H., Chaouachi, A., Chahed, H., Ferchichi, S., Kallel, C., Chamari, K., Souissi, N. (2012). Effect of short-term maximal exercise on biochemical markers of muscle damage, total antioxidant status, and homocysteine levels in football players. *Asian J Sports Med. 3*, 239–246. doi: 10.5812/asjsm.34544.

Jaffe, M. (1886). About the precipitate which picric acid produces in normal urine and about a new reaction of creatinine. *Journal of Physiological Chemistry, 10*(5), 391-400. https://doi.org/10.1515/bchm1.1886.10.5.391

Jameson, J. L., Fauci, A. S., Kasper, D. L., Hauser, S. L., Longo, D. L., & Loscalzo, J. (2018). *Harrison's Principles of Internal Medicine (20ª ed.).* McGraw-Hill Education.

Karila, T. A., Sarkkinen, P., Marttinen, M., Seppälä, T., Mero, A., & Tallroth, K. (2008). Rapid weight loss decreases serum testosterone. *International journal of sports medicine, 29*(11), 872–877. https://doi.org/10.1055/s-2008-1038604

Kawamura, T., & Muraoka, I. (2018). Exercise-Induced Oxidative Stress and the Effects of Antioxidant Intake from a Physiological Viewpoint. *Antioxidants (Basel, Switzerland), 7*(9), 119. https://doi.org/10.3390/antiox7090119

Kivlighan, K. T., & Granger, D. A. (2006). Salivary alpha-amylase response to competition: relation to gender, previous experience, and attitudes. *Psychoneuroendocrinology, 31*(6), 703–714. https://doi.org/10.1016/j.psyneuen.2006.01.007

Larsson, A., Hassan, M., Ridefelt, P., & Axelsson, J. (2009). Circadian variability of bilirubin in healthy men during normal sleep and after an acute shift of sleep. *Chronobiology international, 26*(8), 1613–1621. https://doi.org/10.3109/07420520903398534

Lewis, N. A., Simpkin, A. J., Moseley, S., Turner, G., Homer, M., Redgrave, A., Pedlar, C. R., & Burden, R. (2020). Increased Oxidative Stress in Injured and Ill Elite International Olympic Rowers. *International journal of sports physiology and performance, 15*(5), 625–631. https://doi.org/10.1123/ijspp.2019-0425

Lui, P. P. Y., Zhang, X., Yao, S., Sun, H., & Huang, C. (2022). Roles of Oxidative Stress in Acute Tendon Injury and Degenerative Tendinopathy-A Target for Intervention. *International journal of molecular sciences, 23*(7), 3571. https://doi.org/10.3390/ijms23073571

Meng, Q., & Su, C.-H. (2024). The Impact of Physical Exercise on Oxidative and Nitrosative Stress: Balancing the Benefits and Risks. *Antioxidants, 13*(5), 573. https://doi.org/10.3390/antiox13050573

Nikouei, M., Cheraghi, M., Ghaempanah, F. et al. (2024) The association between bilirubin levels, and the incidence of metabolic syndrome and diabetes mellitus: a systematic review and meta-analysis of cohort studies. *Clin Diabetes Endocrinol 10*, (1). https://doi.org/10.1186/s40842-023-00159-0

Nikouei, M., Cheraghi, M., Ghaempanah, F. et al. (2024). The association between bilirubin levels, and the incidence of metabolic syndrome and diabetes mellitus: a systematic review and meta-analysis of cohort studies. Clin Diabetes Endocrinol 10, (1). https://doi.org/10.1186/s40842-023-00159-0

Palacios, G., Pedrero-Chamizo, R., Palacios, N., Maroto-Sánchez, B., Aznar, S., González-Gross, M., (2015). Biomarkers of physical activity and exercise. *Nutricion hospitalaria, 31*, Suppl 3, 237–244. https://doi.org/10.3305/nh.2015.31.sup3.8771

Pelizzo, P., Stebel, M., Medic, N., Sist, P., Vanzo, A., Anesi, A., Vrhovsek, U., Tramer, F., & Passamonti, S. (2023). Cyanidin 3-glucoside targets a hepatic bilirubin transporter in rats. *Biomedicine & pharmacotherapy, 157*, 114044. https://doi.org/10.1016/j.biopha.2022.114044

Pihut, M., Dziurkowska, E., Wisniewska, G., Szewczyk, M., & Bieganska, J. (2015). Evaluation of the saliva cortisol levels in patients under prosthetic treatment due to functional disorders of the masticatory organ. *Journal of physiology and pharmacology: an official journal of the Polish Physiological Society, 6*6(1), 149–154.

Powers, S. K., Deminice, R., Ozdemir, M., Yoshihara, T., Bomkamp, M. P., & Hyatt, H. (2020). Exercise-induced oxidative stress: Friend or foe?. *Journal of sport and health science, 9*(5), 415–425. https://doi.org/10.1016/j.jshs.2020.04.001

Salvador, A., Suay, F., González-Bono, E., & Serrano, M. A. (2003). Anticipatory cortisol, testosterone and psychological responses to Judo competition in young men. *Psychoneuroendocrinology, 28*(3), 364–375. https://doi.org/10.1016/s0306-4530(02)00028-8

Schwertner, H.A., Vitek, L. (2008). Gilbert syndrome, UGT1A1*28 allele, and cardiovascular disease risk: possible protective effects and therapeutic applications of bilirubin. *Atherosclerosis, 198*, 1-11. doi: 10.1016/j.atherosclerosis.2008.01.001.

Sist, P., & Urbani, R. (2022). Non-invasive approach for the assessment of oxidative stress after intense Judo activities. *Scientific Journal of Sport and Performance, 1*(3), 204-219. https://doi.org/10.55860/WMAW9421

Sist, P., Tramer, F., Bandiera, A., Urbani, R., Redenšek Trampuž, S., Dolžan, V., & Passamonti, S. (2023). Nanoscale Bilirubin Analysis in Translational Research and Precision Medicine by the Recombinant Protein HUG. *International journal of molecular sciences, 24*(22), 16289. https://doi.org/10.3390/ijms242216289

Sist, P., Urbani, R., Tramer, F., Bandiera, A., & Passamonti, S. (2025). The HELP-UnaG Fusion Protein as a Bilirubin Biosensor: *From Theory to Mature Technological Development. Molecules, 30*(3), 439. https://doi.org/10.3390/molecules30030439

Squillacioti, G., Guglieri, F., Colombi, N., Ghelli, F., Berchialla, P., Gardois, P., & Bono, R. (2021). Non-Invasive Measurement of Exercise-Induced Oxidative Stress in Response to Physical Activity. A Systematic Review and Meta-Analysis. *Antioxidants, 10*(12), 2008. https://doi.org/10.3390/antiox10122008

Vítek, L., & Ostrow, J. D. (2009). Bilirubin chemistry and metabolism; harmful and protective aspects. *Current pharmaceutical design, 15*(25), 2869–2883. https://doi.org/10.2174/138161209789058237

Wang, F., Wang, X., Liu, Y., & Zhang, Z. (2021). Effects of Exercise-Induced ROS on the Pathophysiological Functions of Skeletal Muscle. *Oxidative medicine and cellular longevity*, 3846122. https://doi.org/10.1155/2021/3846122

Witek, K., Ścisłowska, J., Turowski, D., Lerczak, K., Lewandowska-Pachecka, S,& Pokrywka, A. (2017). Total bilirubin in athletes, determination of reference range. *Biology of Sport, 34*(1), 45-48. https://doi.org/10.5114/biolsport.2017.63732

Woronyczová, J., Nováková, M., Leníček, M. et al. (2022). Serum Bilirubin Concentrations and the Prevalence of Gilbert Syndrome in Elite Athletes. *Sports Med - Open 8*, (84) https://doi.org/10.1186/s40798-022-00463-6

Yamaguchi, K., Okuda, K., Yonemitsu, H., Tsukada, Y., & Shigeta, H. (1975). Cyclic premenstrual unconjugated hyperbilirubinemia. Report of two cases. *Annals of internal medicine, 83*(4), 514–517. https://doi.org/10.7326/0003-4819-83-4-514

Zuo, L, Huang, J., Zhang, H., et al. (2022). Dose-Response Association Between Bilirubin and Cardiovascular Disease: A Systematic Review and Meta-analysis. *Angiology, 73*(10):911-919. doi:10.1177/00033197211059693

Article history
Received: 7 April, 2026
Accepted: 20 May, 2026