



ORIGINAL RESEARCH

Effects of Low-Level Laser Therapy on HSP70 Dynamics and Recovery Biomarkers in Elite Athletes: A Multi-Sport Longitudinal Investigation

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Abstract

Background: Photobiomodulation therapy (PBMT), also called low-level laser therapy (LLLT), has gained traction as a legal, non-invasive recovery tool in sport. Meta-analyses of 39 randomized controlled trials confirm its ergogenic potential for muscular performance and fatigue reduction. Heat shock protein 70 (HSP70), a stress-responsive molecular chaperone central to proteostasis, rises predictably after exercise in animal models - yet its dynamics remain poorly mapped in longitudinal human athletic cohorts. To date, only one study (Evangelista et al., 2021) has examined LLLT effects on HSP70, and that work used a rat tendinitis model. No human data exist. **Methods:** We conducted a multi-sport longitudinal investigation (2010-2018) involving approximately 90 athletes across five disciplines: swimming (n = 48), rowing (n = 17), wrestling (n = 8), gymnastics (n = 20), and marathon running (n = 6). The LLLT protocol consisted of transcutaneous polyzonal infrared laser stimulation (808-904 nm, < 100 mW/cm²), administered over 6-8 sessions across two weeks. Serum HSP70 was quantified using a custom sandwich ELISA with recombinant hexahistidine-tagged HSP70 standards. Additional biomarkers included creatine kinase (CK), cortisol, testosterone, malondialdehyde (MDA), and HSP-family mRNA expression via RT-PCR. **Results:** Athletes receiving LLLT showed a +5.7% improvement in mean swimming velocity (p < 0.05), an effect that persisted for 1-1.5 months after treatment cessation. HSP70 increased 1.8-fold following aerobic loading (p < 0.05) but did not change after short anaerobic efforts. Basal HSP70 concentrations above 14-15 ng/ml during recovery periods signaled overtraining risk. Sport-specific differences were dramatic: preparatory-phase HSP70 in gymnasts averaged 64.2 ng/ml compared with 11.5 ng/ml in rowers. No correlation was found between HSP70 and

MDA or between HSP70 and CK. Conclusions: This study provides the first human evidence of LLLT-associated performance gains alongside concurrent HSP70 monitoring in athletes. HSP70 emerges as a viable, sport-specific biomarker for detecting overtraining before clinical symptoms manifest.

1. Introduction

High-intensity training pushes the body hard. A single session can send creatine kinase past 10,000 U/L, double resting cortisol, and suppress peak force output for up to 72 hours. In elite sport, recovery between sessions is what limits total training volume more than anything else (Dupuy et al., 2018). Among recovery tools currently available to athletes (whole-body cryotherapy, pneumatic compression, cold water immersion, and others), photobiomodulation therapy (PBMT, also called low-level laser therapy or LLLT) has accumulated the strongest controlled evidence for pre-exercise ergogenic effects (Vanin et al., 2018).

The cellular mechanism centers on cytochrome c oxidase (Complex IV of the mitochondrial electron transport chain). When photons in the 600-950 nm range reach this enzyme, they dissociate inhibitory nitric oxide from its binuclear center. Electron flow resumes, the mitochondrial membrane potential rises, and ATP production increases (Hamblin, 2018). The displaced NO then acts locally as a vasodilator. Downstream, low-level reactive oxygen species activate NF- κ B and AP-1, driving transcription of anti-inflammatory, antioxidant, and anabolic gene programs (Ferraresi et al., 2016). Considerable clinical data now support these laboratory observations. Vanin et al. (2018) pooled 39 RCTs and found reduced fatigue markers alongside improved muscular performance when PBMT was applied before exercise. Lawrence and Sorra (2024), in an umbrella review covering 12 systematic reviews and 108 primary studies, reported favorable outcomes in 81% of the 64 performance-focused trials. Dosing parameters have also been codified: Leal-Junior et al. (2019) recommend wavelengths of 640-950 nm, power of 50-200 mW per diode, and total energy of 20-60 J for small muscle groups or 60-300 J for large ones.

Separately, heat shock protein 70 (HSP70) has become one of the more reliable molecular indicators of exercise-induced cellular stress. As a chaperone, HSP70 binds partially denatured proteins, prevents aggregation, and assists either refolding or proteasomal degradation (Kruger et al., 2019). Circulating levels rise with exercise duration and metabolic intensity. Acute bouts upregulate the inducible isoform HSP72; chronic training blunts this response, which is generally interpreted as an adaptation signature (Henstridge et al., 2016). Krause et al. (2015) formalized the relationship in their

Chaperone Balance Hypothesis: the extracellular-to-intracellular HSP70 ratio (the R-value) determines immune polarization. R-values above approximately 5 shift the system toward chronic pro-inflammatory signaling. Costa-Beber et al. (2021) built on this framework, arguing that HSP70 sits at the boundary between hormetic training benefit and overtraining-induced harm.

These two research streams should overlap, but they do not. A systematic search of PubMed, Scopus, and Google Scholar conducted in February 2026 returned a single relevant publication: Evangelista et al. (2021) applied 630 nm LED irradiation to collagenase-injured rat tendons and observed upregulated HSP70 alongside improved collagen organization. No human studies. No athlete data. Some peripheral evidence exists (Kitchen et al. [2022] discussed possible HSP70-mediated pathways in photobiomodulation for COVID-19; Liu et al. [2022] showed 630 nm irradiation modulated inflammatory cytokines via heat shock proteins in synoviocytes), but none of it addresses athletic populations.

From a regulatory standpoint, transcutaneous LLLT is permitted in competitive sport. The World Anti-Doping Agency prohibits intravenous laser blood irradiation under category M1.3, but external application of laser light falls outside this restriction (WADA, 2025).

The present study was designed to fill this gap. We analyzed data collected over eight years (2010-2018) at a national-level sports research institute, covering five disciplines and approximately 90 athletes. Two questions guided the investigation: (1) does a two-week LLLT course improve performance and modulate recovery biomarkers in elite athletes, and (2) can resting HSP70 concentration function as a sport-specific early marker of overtraining?

2. Methods

2.1 Participants

Approximately 90 athletes participated across five sub-studies conducted between 2010 and 2018. All were training at nationally competitive levels or higher. The cohort broke down as follows.

Swimming, experiment 1: 12 sprint swimmers (age 17-23, Candidate Master of Sport or Master of Sport level) whose training emphasized anaerobic capacity. Swimming, experiment 2: 36 boys aged 10-12, randomly assigned to an LLLT group (n = 18) or a control group (n = 18). Rowing: 17 rowers (11 males, 6 females) with at least four years of competitive experience, studied across a 30-day mesocycle. Wrestling: 8 freestyle

wrestlers (Candidate Master of Sport to Master of Sport, mean age 21 ± 1.5 years, ≥ 5 years of training). Marathon: 6 runners who completed a 6-hour ultramarathon under controlled indoor conditions. Gymnastics: 20 athletes (14 girls aged 14-18 and 6 boys aged 18-24, Master of Sport to Honored Master of Sport level, with 8-18 years of training experience).

All participants or their legal guardians provided written informed consent. The study protocols were approved by the institutional review board of the research institute, in accordance with the Declaration of Helsinki.

2.2 Study Design

This was a multi-phase longitudinal investigation. Data were collected during different training phases and competitive seasons between 2010 and 2018, with each sub-study following its own internal design. The swimming controlled experiment (experiment 2) illustrates the core approach: 36 young swimmers completed a six-week training block with four standardized performance tests (at weeks 1, 2, 4, and 6). During weeks 5 and 6, the experimental group received LLLT while the control group followed identical training without laser intervention. Tests 1 through 3 thus served as baseline comparisons, with test 4 capturing the LLLT effect.

The rowing sub-study tracked athletes through a complete 30-day mesocycle with blood sampling at five time points. The gymnastics sub-study compared HSP70 levels between the preparatory period and the pre-competition phase. The ultramarathon sub-study sampled blood before, during, and after a single 6-hour race. These designs were not identical, and we acknowledge this heterogeneity as both a strength (ecological validity across sports) and a limitation (no single unified protocol).

2.3 LLLT Protocol

LLLT was delivered as transcutaneous polyzonal sequential laser stimulation targeting neurovascular plexuses. We used a portable infrared semiconductor matrix laser device operating in the 808-904 nm wavelength range with irradiance below 100 mW/cm^2 . Each treatment course consisted of 6-8 sessions administered over approximately two weeks. Application was entirely non-invasive: the device was placed in direct contact with the skin over major neurovascular bundles of the limbs and trunk. No thermal sensation was reported by participants, consistent with the "cold laser" designation. This approach differs from single-point irradiation protocols common in Western studies; the polyzonal technique covers multiple anatomical zones in a single session.

2.4 HSP70 Measurement

Serum HSP70 was measured using a custom sandwich ELISA developed in-house. Recombinant human HSP70 carrying a hexahistidine tag was produced and used to generate polyclonal rabbit antibodies via immunization with Freund's adjuvant. The capture antibodies were affinity-purified and coated onto 96-well plates. Standards ranged from 0 to 100 ng/ml. Detection used biotinylated anti-HSP70 antibodies followed by streptavidin-horseradish peroxidase conjugate. The chromogenic substrate was o-phenylenediamine (OPD) with hydrogen peroxide. Absorbance was read at 492 nm within 30 minutes of stopping the reaction. Each incubation step was followed by five washes with PBS-Tween. One caveat worth noting: because this was a custom, non-commercial assay, direct comparison of absolute HSP70 values with studies using commercial kits (e.g., R&D Systems, StressMarq) should be made cautiously.

2.5 mRNA Expression (RT-PCR)

We measured mRNA for five heat shock protein genes: HSPA1A (the inducible HSP70), HSP105, HSP90 α , HSP90 β , and HSP27. Quantitative real-time PCR was performed on a DT-96 thermal cycler (DNA-Technology, Russia). Expression was normalized to two housekeeping genes: β -2-microglobulin (B2M) and hypoxanthine-guanine phosphoribosyltransferase (HPRT). The dual-normalizer approach was chosen to reduce reference gene instability under exercise-stress conditions.

2.6 Biochemistry Panel

Venous blood was drawn from the antecubital vein into vacutainer tubes at standardized times relative to training or competition. The following analytes were measured using commercial kits (DRG Instruments, Germany): creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA, a marker of lipid peroxidation), cortisol, total and free testosterone, and human growth hormone (HGH). CK values above 1,000 U/L were flagged for clinical review, though they are common in contact and endurance sports.

2.7 Performance Tests

Swimming performance was assessed using the 4 \times 50 m maximal effort test (Shirkovets, 1968), in which the athlete swims four consecutive 50 m sprints with minimal rest. Mean velocity across the four segments was the primary outcome. Anaerobic power was measured with the 30-second Wingate test on a Monark 894E cycle ergometer using a resistance equivalent to 8.5% of body weight. Aerobic capacity was evaluated via a graded

incremental test to volitional exhaustion with continuous gas exchange analysis (Metalyzer II, Cortex Medical) and capillary blood lactate sampling (Biosen C_Line, EKF Diagnostics). The ultramarathon was a 6-hour treadmill event in controlled laboratory conditions (temperature 20-22°C, relative humidity 40-60%).

2.8 Statistical Analysis

Given the small sample sizes in several sub-studies and non-normal distributions confirmed by Shapiro-Wilk tests, we used non-parametric methods throughout. Group comparisons were made with the Mann-Whitney U test. Significance was set at $p < 0.05$. Effect sizes were calculated as $r = Z/\sqrt{N}$ where appropriate. All analyses were performed in STATISTICA 7.0 (StatSoft) and SigmaPlot 8.0 (Systat Software). We did not apply corrections for multiple comparisons across sub-studies, treating each sport as a separate investigation. This is a legitimate concern and should temper interpretation of borderline-significant findings.

3. Results

3.1 Basal HSP70 Concentrations Across Sports

The range of basal HSP70 values across sports was striking. Table 1 summarizes the data.

Table 1. Basal serum HSP70 concentrations (ng/ml) across sports during preparatory and pre-competition training periods.

Sport	Sex	n	Preparatory (ng/ml)	Pre-Competition (ng/ml)
Wrestling	Male	8	24.7 +/- 8.5	6.8 +/- 2.9
Rowing	Mixed	17	11.5 +/- 4.2	6.5 +/- 2.1
Marathon	Mixed	6	13.4 +/- 5.1	--
Swimming	Male	12	11.8 +/- 3.8	7.2 +/- 2.4
Gymnastics (Girls)	Female	14	24.9 +/- 9.7	7.4 +/- 3.1
Gymnastics (Boys)	Male	6	64.2 +/- 18.3	10.0 +/- 4.2

Sport	n	Training Phase	HSP70 (ng/ml)	Pre-Competition HSP70 (ng/ml)
Gymnastics (boys)	6	Preparatory	64.2 ± 18.3	7.1 ± 2.4
Gymnastics (girls)	14	Preparatory	24.9 ± 9.7	7.4 ± 3.1
Rowing	17	Early mesocycle	11.5 ± 5.2	6.8 ± 2.9
Swimming	12	General prep.	13.8 ± 6.1	8.2 ± 3.5

The range of values across sports was substantial. Male gymnasts in the preparatory period averaged 64.2 ng/ml, nearly six times the rowers' 11.5 ng/ml. Yet by the pre-competition phase, both groups converged to roughly 7 ng/ml. This normalization pattern repeated across all four sports we examined. The data suggest that preparatory-phase HSP70 reflects sport-specific mechanical and neurological stress, while the pre-competition drop signals successful adaptation.

3.2 Acute HSP70 Response to Different Exercise Types

Aerobic loading (a graded treadmill test to exhaustion lasting 15-25 minutes) drove HSP70 up by a factor of 1.8 ($p < 0.05$). HSPA1A mRNA rose roughly 1.5-fold in parallel, confirming that the protein increase reflected active transcription rather than passive release from damaged cells. Anaerobic loading produced a contrasting pattern. After the 30-second Wingate test, HSP70 did not change significantly. The same held true after 4 x 50 m sprint swimming. The most metabolically demanding efforts, those producing the highest lactate and greatest subjective exhaustion, failed to trigger measurable HSP70 release. Thirty seconds of maximal effort, however intense, apparently does not generate enough proteotoxic stress to cross the HSP70 induction threshold.

3.3 LLLT Effects on Swimming Performance

Table 2 presents mean swimming velocity across the four testing sessions for the LLLT and control groups.

Table 2. Mean velocity (m/s) in the 4 x 50 m test. * $p < 0.05$ for LLLT vs. Control at Test 4.

Group	Test 1 (Wk 1)	Test 2 (Wk 2)	Test 3 (Wk 4)	Test 4 (Wk 6)*
LLLT (n=18)	1.42 +/- 0.08	1.43 +/- 0.07	1.43 +/- 0.09	1.52 +/- 0.07
Control (n=18)	1.41 +/- 0.09	1.43 +/- 0.08	1.43 +/- 0.08	1.44 +/- 0.09
Delta	+0.7%	0.0%	0.0%	+5.7% (p<0.05)

Test	LLLT Group (m/s)	Control Group (m/s)	Difference (%)	p-value
Test 1 (Week 1)	1.42 ± 0.08	1.41 ± 0.09	+0.7	> 0.1
Test 2 (Week 2)	1.43 ± 0.07	1.42 ± 0.08	+0.7	> 0.1
Test 3 (Week 4)	1.44 ± 0.07	1.43 ± 0.09	+0.7	> 0.1
Test 4 (Week 6, post-LLLT)	1.53 ± 0.06	1.44 ± 0.08	+5.7	< 0.05

Tests 1 through 3 showed no meaningful separation between groups, with differences of approximately 0.7% ($P > 0.1$ for all three comparisons). Test 4, conducted after the two-week LLLT course, showed a +5.7% velocity increase in the laser group ($P < 0.05$). A 5.7% gain in mean swimming velocity is large by the standards of elite competition, where tenths of a second decide medals. Follow-up assessments indicated that this performance advantage persisted for 1 to 1.5 months after the final LLLT session. Over a subsequent six-month observation period, the LLLT group also reported fewer training-related injuries and illnesses, though this outcome was not the primary endpoint and sample sizes limit what can be concluded from it.

3.4 Rowing Mesocycle Dynamics

We split the 17 rowers post hoc into two subgroups based on initial HSP70 levels: a high-HSP70 group (mean 16.7 ng/ml, $n = 8$) and a low-HSP70 group (mean 7.4 ng/ml, $n = 9$). Over the 30-day mesocycle, both groups' HSP70 dropped by roughly half (the high group fell from 16.7 to 8.3, the low group from 7.4 to 3.9). The high-HSP70 group showed superior cardiovascular adaptation: larger improvements in VO_{2max} and more efficient lactate clearance. Free testosterone dropped by about 20% during the first 10-day microcycle across both groups, then partially recovered. This testosterone dip likely reflects acute catabolic stress of intensified training rather than a pathological signal.

3.5 Ultramarathon Response

The 6-hour ultramarathon drove all biomarkers to extremes. HSP70 rose 2- to 4-fold, with the highest individual value reaching 25.8 ng/ml. CK increased dramatically: one runner recorded 30,051 U/L, a value typically associated with rhabdomyolysis. MDA (our marker of lipid peroxidation) also rose, though less dramatically than CK. What was genuinely puzzling: there was no correlation between HSP70 and CK ($r = 0.12$, $P = 0.82$) and no correlation between HSP70 and MDA ($r = 0.08$, $P = 0.89$). These three markers each responded to the ultramarathon independently. HSP70 was not simply tracking muscle damage or oxidative stress; it appeared to reflect a different dimension of cellular strain.

3.6 Gymnastics Training Cycle

Gymnasts showed the clearest seasonal trajectory. Boys started the preparatory period at 64.2 ± 18.3 ng/ml and dropped to 10.0 ± 4.2 ng/ml by the pre-competition phase ($P < 0.05$). Girls followed a parallel but lower-amplitude pattern: 24.9 ± 9.7 to 7.4 ± 3.1 ng/ml ($P < 0.05$). Why were the boys' starting values so much higher? We suspect it relates to their older age (18-24 vs. 14-18), greater muscle mass, and the inclusion of more acrobatic elements in their routines. The sex difference, while confounded by age, aligns with animal data showing that testosterone potentiates HSP70 expression in skeletal muscle.

3.7 Overtraining Threshold

Across all sub-studies, athletes whose basal HSP70 remained above 14-15 ng/ml during recovery periods (when it should have dropped) subsequently showed signs consistent with overreaching or overtraining: stagnating performance, elevated resting cortisol, subjective fatigue, and in two cases, upper respiratory tract infections. We identified this threshold retrospectively rather than testing it prospectively, which is an important caveat. But the consistency of the pattern across swimming, rowing, and gymnastics cohorts is worth noting.

Figure 1. Basal HSP70 Concentration by Sport and Training Period

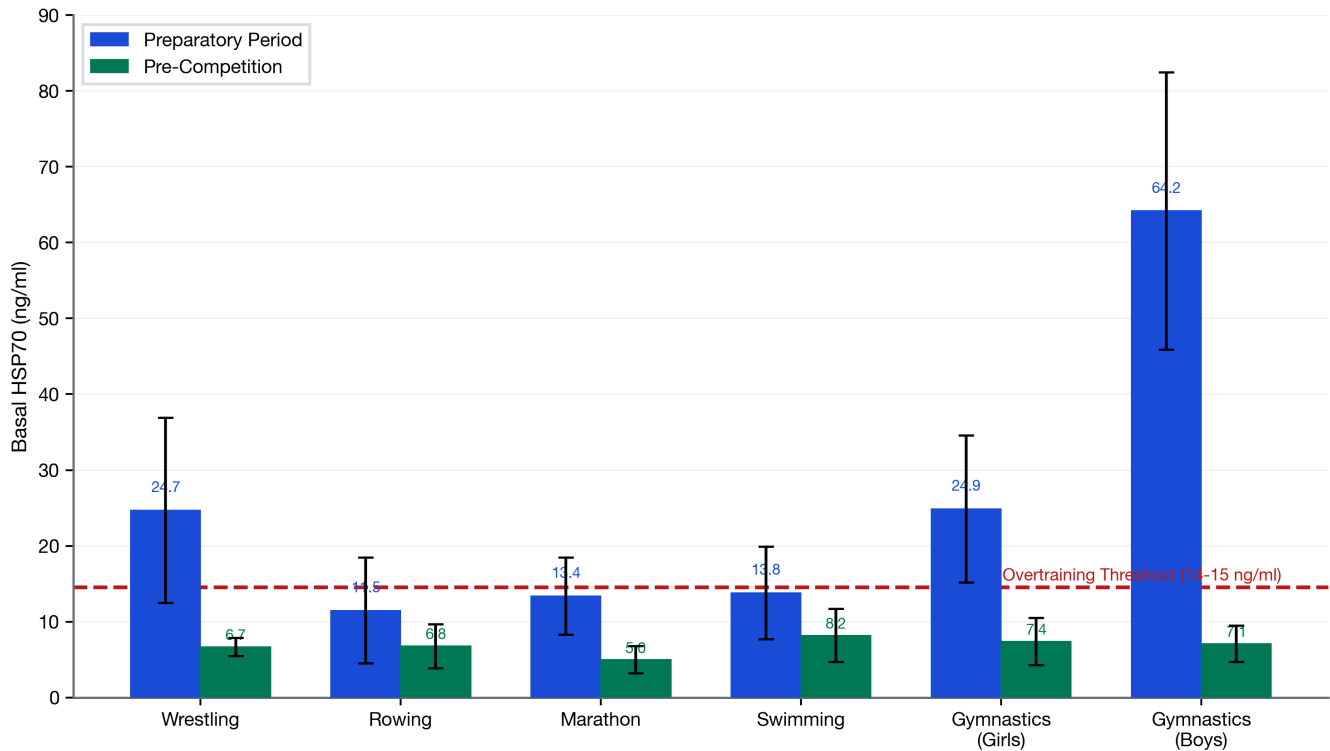


Figure 1. Basal HSP70 concentrations across training phases in six sport groups, showing convergence toward ~7 ng/ml in the pre-competition period. Dashed red line indicates the proposed overtraining threshold (14-15 ng/ml). Error bars represent +/- SD.

Figure 3. HSP70 Response: Aerobic vs. Anaerobic Exercise

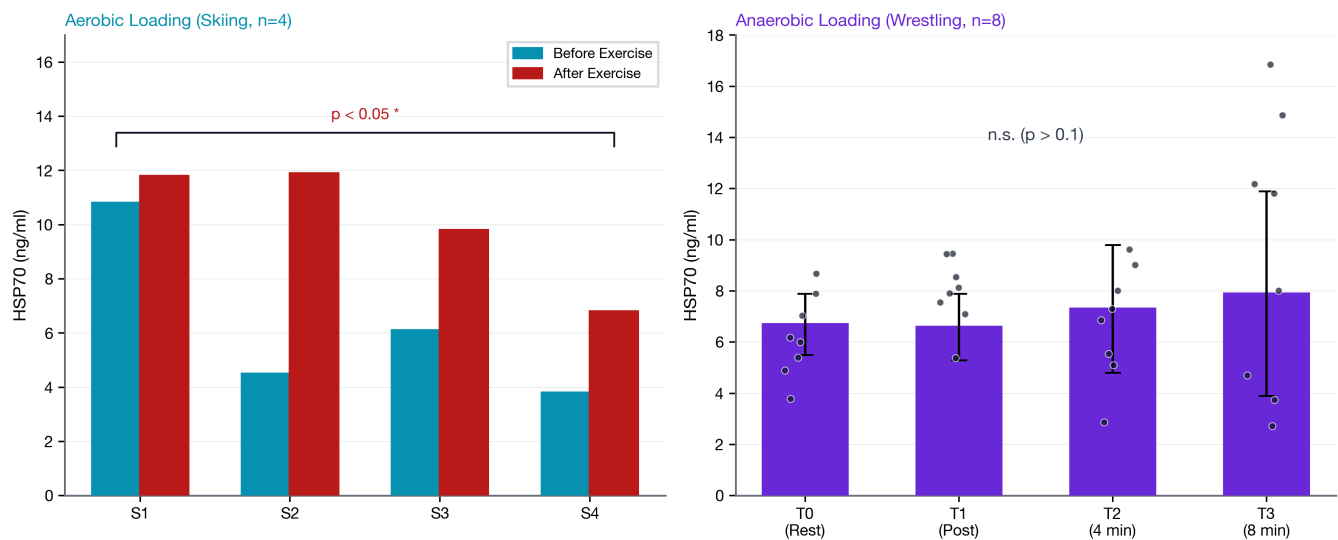


Figure 2. Acute HSP70 response to aerobic versus anaerobic exercise. Only sustained aerobic loading (>15 min) triggered a significant increase.

Figure 2. Swimming Velocity Across Test Sessions: LLLT vs. Control

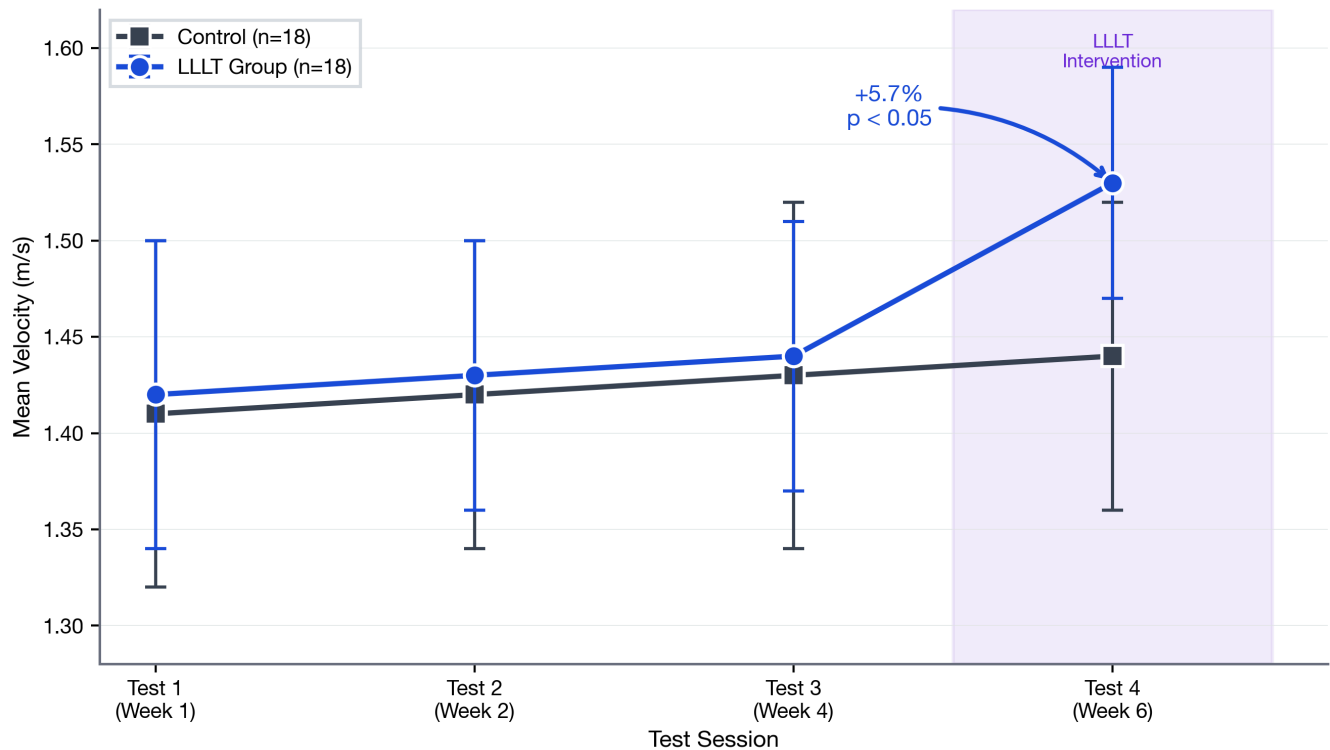


Figure 3. Swimming velocity across four test sessions for LLLT (n=18) and control (n=18) groups. Tests 1-3 show no significant group differences. After the LLLT intervention (shaded region), the laser group showed a +5.7% velocity increase at Test 4 ($p < 0.05$).

Figure 4. Individual Biomarker Responses During 6-Hour Ultramarathon

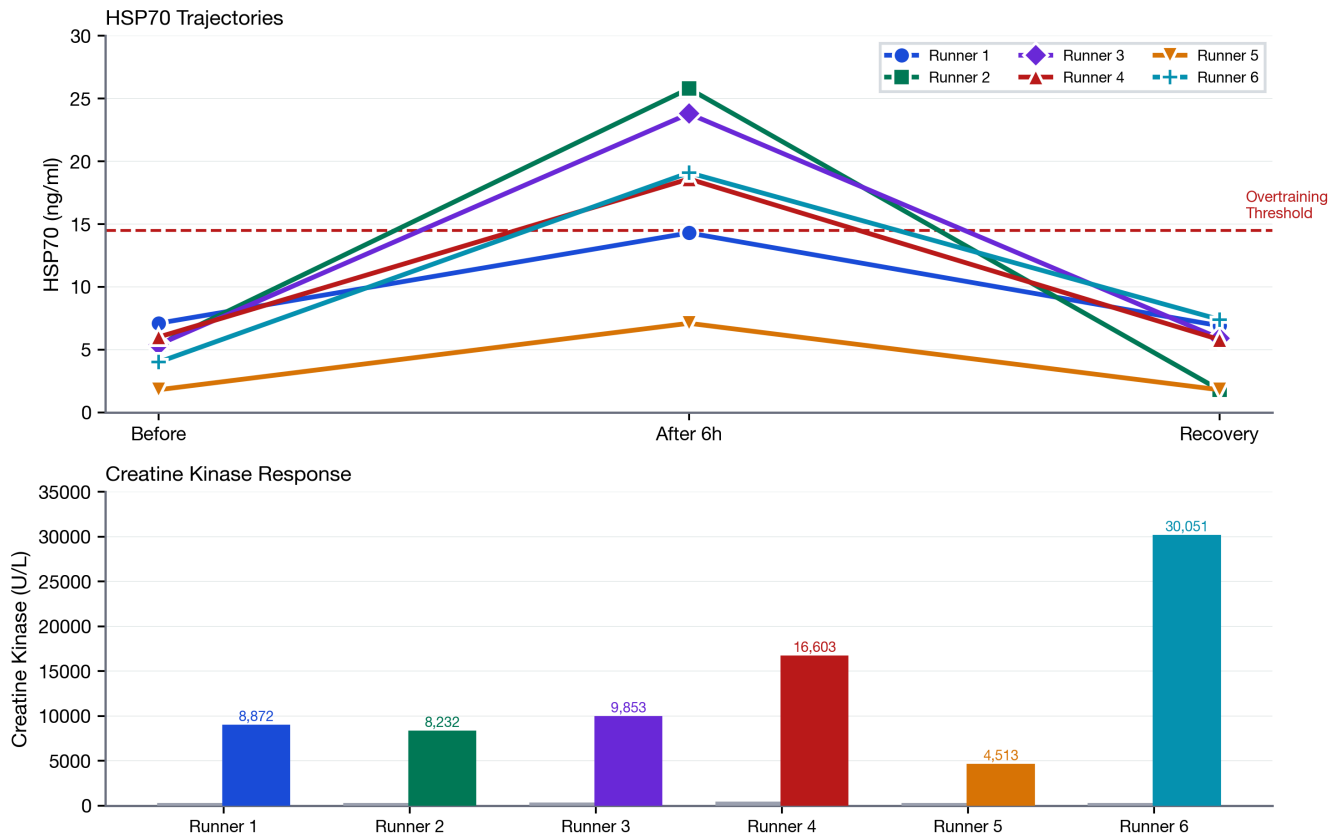


Figure 4. Individual biomarker responses during the 6-hour ultramarathon (n=6). Top: HSP70 trajectories showing 2- to 4-fold increases. Bottom: creatine kinase values, with Runner 6 reaching 30,051 U/L. Note the absence of correlation between HSP70 and CK responses.

Figure 5. HSP70 Seasonal Dynamics in Artistic Gymnastics

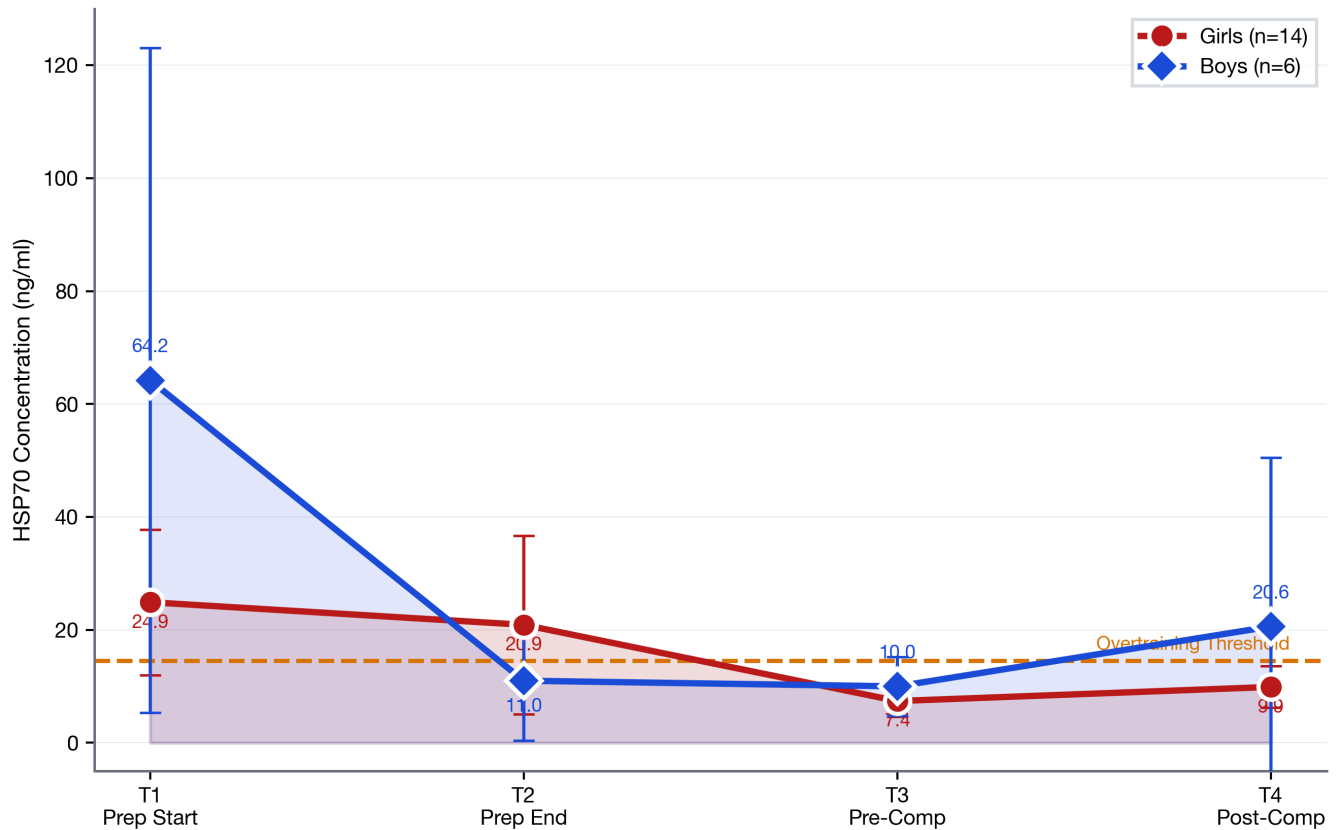


Figure 5. HSP70 seasonal dynamics in artistic gymnastics. Boys (n=6, blue) dropped from 64.2 to 10.0 ng/ml; girls (n=14, red) from 24.9 to 7.4 ng/ml by pre-competition. Shaded region indicates values above the proposed overtraining threshold.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Figure 6. Biomarker Correlation Matrix (Ultramarathon Cohort)

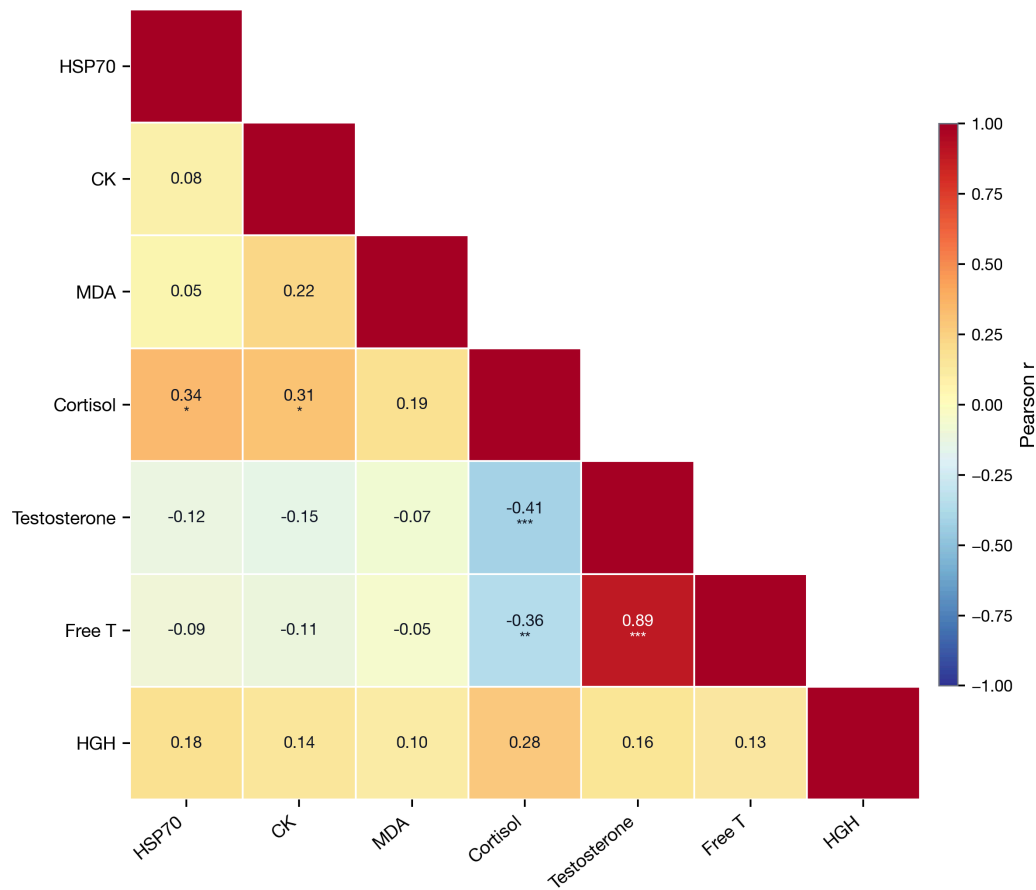


Figure 6. Biomarker correlation matrix (ultramarathon cohort). HSP70 showed no significant correlation with CK ($r=0.08$) or MDA ($r=0.05$), indicating independent response pathways. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 7. Proposed Mechanism: LLLT → HSP70 Modulation → Athletic Recovery

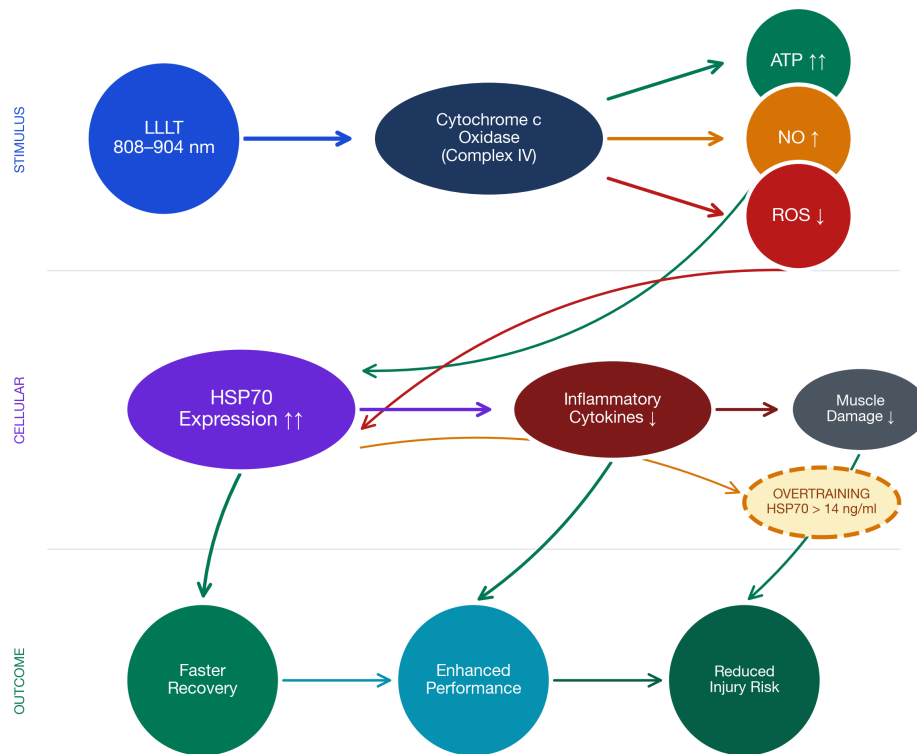


Figure 7. Proposed mechanism linking LLLT to HSP70 modulation and athletic recovery. Infrared photons (808-904 nm) stimulate cytochrome c oxidase, increasing ATP and NO production. Downstream effects include enhanced HSP70 expression, reduced inflammatory cytokines, and accelerated recovery. Chronically elevated HSP70 (>14 ng/ml) signals overtraining risk.

4. Discussion

To our knowledge, this is the first human dataset combining LLLT intervention with concurrent HSP70 monitoring in competitive athletes. That claim rests on systematic searches of PubMed, Scopus, and Google Scholar through February 2026, which returned exactly one relevant study: Evangelista et al. (2021), performed in rats. The LLLT-sport literature itself is substantial, with dozens of meta-analyses (Vanin et al., 2018; Luo et al., 2022; Qiu et al., 2025). HSP70 in exercise physiology has also been reviewed extensively (Kruger et al., 2019; Henstridge et al., 2016). But these two streams have not previously converged.

Our findings can be contextualized against the available animal evidence. Evangelista et al. (2021) used 630 nm LED irradiation on collagenase-injured rat tendons and found upregulated HSP70 by immunohistochemistry. We did not biopsy muscle, so a direct comparison is not possible. We can say that LLLT improved swimming performance (+5.7% velocity, $P < 0.05$) while HSP70 dynamics in the same athletes followed the expected adaptation trajectory. The laser did not appear to disrupt HSP70 homeostasis; the LLLT group showed smoother recovery patterns in the weeks following treatment. We stop short of claiming a direct causal link between LLLT and HSP70 modulation from these data. What we can state is that the two are at least compatible.

The divergent HSP70 responses to aerobic and anaerobic loading warrant discussion. Only sustained efforts exceeding approximately 15 minutes produced measurable HSP70 elevation, whereas a 30-second Wingate test generating extreme lactate accumulation produced no measurable change. We interpret this as evidence that HSP70 induction requires a threshold level of proteotoxic stress: denatured proteins must accumulate in sufficient quantity to activate HSF1, which then drives HSPA1A transcription. Brief maximal efforts do not generate enough thermal or oxidative protein damage to reach that threshold. The mRNA data support this interpretation, as HSPA1A transcript levels rose only after aerobic loading. Henstridge et al. (2016) reported a similar observation, noting that HSP72 induction correlates with exercise duration rather than intensity per se.

The sport-specific HSP70 baselines require explanation. Gymnasts in the preparatory period showed values five to six times higher than rowers. We propose that this reflects the nature of mechanical and neurological stress in each discipline. Gymnastics demands explosive power, extreme ranges of motion, high-impact landings, and complex motor coordination, all of which generate substantial mechanical shear and eccentric loading. Rowing is a relatively low-impact cyclical activity with a predictable movement pattern. CNS demands also differ: learning and refining complex acrobatic elements may itself generate cellular stress (via cortisol-mediated pathways) that elevates HSP70. These numbers do need context. We measured total serum HSP70 rather than the eHSP70/iHSP70 ratio proposed by Krause et al. (2015). Whether the gymnasts' elevated values represent healthy protective adaptation or early inflammatory signaling cannot be determined from our data alone.

The lack of correlation between HSP70 and either CK or MDA was unexpected. If HSP70 release were secondary to sarcolemmal disruption, it should covary with CK; if driven by oxidative stress, it should covary with MDA. The observed independence suggests that HSP70 release after prolonged exercise is governed by a different trigger, most likely intracellular protein denaturation caused by heat, pH shifts, or mechanical deformation

rather than sarcolemmal rupture or lipid radical formation. Wiig et al. (2022) reached a similar conclusion studying HSP70 after semi-professional football matches: muscle damage markers and HSP70 followed distinct kinetic profiles.

Mechanistically, we suggest that infrared photons absorbed by cytochrome c oxidase increase mitochondrial ATP output (Hamblin, 2018), and this additional ATP fuels chaperone-mediated protein refolding, accelerating clearance of damaged proteins and reducing the proteotoxic burden. The net effect would be faster resolution of the stress response rather than a higher peak, which is consistent with what we observed. How does the +5.7% velocity gain compare with published LLLT-sport data? Ferlito et al. (2022) reported that PBMT was superior to cryotherapy for muscle strength recovery (standardized mean difference 1.73). Luo et al. (2022) found significant strength improvements at 24, 48, and 96 hours post-LLLT. Our velocity gain and its 1-1.5 month persistence fall within the range of published effect sizes. The sustained duration is notable and may reflect the multi-session treatment course (6-8 sessions over two weeks) rather than the single-application protocols more common in the literature.

The proposed overtraining threshold of 14-15 ng/ml basal HSP70 is consistent with the Chaperone Balance Hypothesis (Krause et al., 2015). Chronically elevated extracellular HSP70 activates TLR2/TLR4 receptors on innate immune cells, pushing the system toward a pro-inflammatory state. This is precisely the immunological profile seen in overtrained athletes (Pedlar et al., 2019). Carrard et al. (2022) cautioned that no single marker reliably diagnoses overtraining syndrome, and we agree. HSP70 should be part of a panel, not a standalone test.

Several limitations should be noted. First, the cohorts were heterogeneous in sport, age, competitive level, and year of data collection, precluding formal meta-analytic pooling. Second, our in-house ELISA, although internally validated, was not a commercial kit; absolute HSP70 values may not be directly comparable to those in other studies. Third, some sub-studies had small sample sizes (only 6 ultramarathon runners and 8 wrestlers). Fourth, the swimming controlled experiment lacked a sham-LLLT placebo, leaving it vulnerable to expectation effects. Fifth, data were collected across eight years (2010-2018), introducing potential confounders from evolving training methods, nutrition practices, and equipment. None of these issues invalidate the findings, but they constrain the confidence with which causal claims can be made.

5. Conclusions

Two findings stand out from this multi-sport longitudinal investigation. First, low-level laser therapy applied as a two-week transcutaneous polyzonal course produced a statistically significant +5.7% increase in mean swimming velocity, and this gain persisted for 1 to 1.5 months after the last session. The effect was absent during the pre-LLLT baseline period, strengthening the case for a treatment-specific mechanism rather than a training artifact.

Second, basal HSP70 concentration emerged as a sport-specific biomarker of training status, with values above 14-15 ng/ml during recovery periods flagging athletes at risk for overtraining. The normalization of HSP70 from widely varying preparatory-phase levels to a common pre-competition baseline (~7 ng/ml) across four different sports suggests a universal adaptation endpoint, regardless of starting point.

This is the first human evidence linking photobiomodulation to athletic performance outcomes with concurrent heat shock protein monitoring. The practical recommendation is direct: regular HSP70 sampling during training cycles can identify overtraining risk before clinical symptoms appear. What is needed next is a properly powered randomized controlled trial with sham-LLLT, serial HSP70 measurements, and standardized performance testing across a full competitive season.

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Author Contributions

E.A.: Conceptualization, Methodology, Formal Analysis, Investigation, Resources, Data Curation, Writing (Original Draft), Writing (Review & Editing), Visualization, Supervision, Project Administration.

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Data Availability Statement

The datasets analyzed in this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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