Integrating circadian biology and resistance training: time-of-day effects on hypertrophy, hormonal flux and the muscle transcriptome in bodybuilders

Integración de la biología circadiana y el entrenamiento de resistencia: efectos de la hora del día sobre la hipertrofia, el flujo hormonal y el transcriptoma muscular en culturistas

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Abstract

Introduction: Circadian rhythms influence muscle metabolism and gene expression, suggesting that training time-of-day may shape hypertrophic and molecular adaptations. Evidence in trained individuals, however, remains limited.

Objective: To compare the effects of morning versus evening resistance training on hypertrophy, performance, endocrine markers, sleep, and skeletal-muscle transcriptomics in bodybuilders.

Methodology: In a randomized parallel-group trial, 112 trained males were assigned to 12 weeks of supervised training either in the morning (07:00–09:00) or evening (17:00–19:00). Primary outcome was change in vastus lateralis CSA (MRI). Secondary outcomes included lean mass (DXA), strength, hormones, sleep (actigraphy), chronotype, and RNA-seq profiling.

Results: Evening training produced a greater VL-CSA increase (+8.2% vs +6.0%; p=0.04) and stronger induction of mTOR- and ribosome-related transcriptional pathways (FDR < 0.05). Chronotype moderated hypertrophic responses.

Discussion: Training time influenced phenotypic and molecular adaptations, with evening sessions eliciting broader anabolic signaling.

Conclusion: Evening resistance training yields modestly greater hypertrophy and distinct transcriptomic responses; aligning training with chronotype may enhance outcomes.

Keywords

Circadian physiology; resistance training hypertrophy; chronotype; skeletal muscle transcriptomics; mTOR and ribosome biogenesis.

Resumen

Introducción: Los ritmos circadianos influyen en el metabolismo muscular y la expresión génica, lo que sugiere que el momento del día en que se entrena puede determinar las adaptaciones hipertróficas y moleculares. Sin embargo, la evidencia en individuos entrenados aún es limitada.

Objetivo: Comparar los efectos del entrenamiento de resistencia matutino versus vespertino sobre la hipertrofia, el rendimiento, los marcadores endocrinos, el sueño y la transcriptómica del músculo esquelético en culturistas.

Metodología: En un ensayo aleatorizado de grupos paralelos, 112 hombres entrenados fueron asignados a 12 semanas de entrenamiento supervisado, ya sea por la mañana (07:00–09:00) o por la tarde (17:00–19:00). El resultado primario fue el cambio en el área de sección transversal (CSA) del vasto lateral (RM). Los resultados secundarios incluyeron la masa magra (DXA), la fuerza, las hormonas, el sueño (actigrafía), el cronotipo y el perfil de ARN mediante secuenciación (RNA-seq). Resultados: El entrenamiento vespertino produjo un mayor incremento en el área de sección transversal del músculo vasto lateral (+8,2 % frente a +6,0 %; p = 0,04) y una inducción más potente de las vías de transcripción relacionadas con mTOR y los ribosomas (FDR < 0,05). El cronotipo moduló las respuestas hipertróficas.

Discusión: El horario de entrenamiento influyó en las adaptaciones fenotípicas y moleculares, y las sesiones vespertinas provocaron una señalización anabólica más amplia.

Conclusión: El entrenamiento de resistencia vespertino produce una hipertrofia ligeramente mayor y respuestas transcriptómicas distintas; alinear el entrenamiento con el cronotipo podría mejorar los resultados.

Palabras clave

Fisiología circadiana; hipertrofia por entrenamiento de resistencia; cronotipo; transcriptómica del músculo esquelético; mTOR y biogénesis de ribosomas.

Introduction

Resistance training (RT) induces increases in muscle size, strength, and metabolic capacity through the coordinated integration of mechanical loading and intracellular signaling pathways. At the cellular level, hypertrophy is governed by mechanotransduction, satellite-cell activation, anabolic hormone sensitivity, and translational regulation via mTORC1 and ribosome biogenesis (Yang et al., 2022; Kim et al., 2019). Traditionally, these processes were viewed as primarily load-dependent and largely independent of temporal context. However, evidence from chronobiology has reshaped this view by revealing that skeletal muscle is a strongly circadian tissue with >20% of its transcriptome exhibiting time-of-day oscillations (Zambon et al., 2003). Core clock components such as BMAL1, PER2, and REV-ERBα modulate metabolism, mitochondrial turnover, protein synthesis, and myogenic signaling, with downstream consequences for muscle plasticity (Hodge et al., 2022). Moreover, systemic circadian influences including daily rhythms in testosterone, cortisol, neuromuscular activation (Nobari et al., 2023), body temperature, and substrate availability create fluctuating internal environments that may alter the responsiveness of muscle to resistance exercise (Chtourou & Souissi, 2012). Exercise itself can shift or reinforce peripheral clock rhythms, acting as a zeitgeber that interacts with endogenous timekeeping mechanisms (Martin & Esser, 2022). Together, these findings suggest that the physiological state of muscle and the endocrine-metabolic milieu into which training is introduced vary across the day, raising the possibility that identical RT stimuli performed at different times may not be biologically equivalent. For athletes whose progress relies on optimizing the cumulative impact of repeated training bouts, understanding these temporal interactions is increasingly important.

Research Problem and Gap

Despite theoretical plausibility and supportive mechanistic evidence, empirical findings from human time-of-day training studies remain mixed. Some trials report superior performance or hypertrophy when training and testing occur at the same circadian time (Sedliak et al., 2009). Others document small or inconsistent differences between morning and evening RT, often constrained by limited sample sizes, heterogeneous protocols, or insufficient control of chronotype and sleep (Grgic et al., 2019). Meta-analyses indicate only modest pooled effects, with large variability attributable to methodological limitations and inadequate biological characterization of participants.

Simultaneously, advances in muscle transcriptomics demonstrate that resistance exercise triggers broad, time-sensitive gene expression programs involving mTOR signaling, ribosomal biogenesis, proteostasis networks, inflammatory cascades, and the molecular clock (Landen et al., 2023; Kim et al., 2019). Yet, studies integrating controlled chrono-exercise interventions with deep molecular phenotyping remain rare, particularly in trained bodybuilders, whose adaptive ceiling is high and in whom small molecular advantages may translate into measurable performance benefits. This constitutes a critical gap: there is limited understanding of how training time interacts with individual chronotype to modulate hypertrophy, endocrine responses, and transcriptomic remodeling.

Aim and Objectives

This randomized, single-blinded controlled trial was designed to address these unresolved questions by directly comparing morning (07:00–09:00) and evening (17:00–19:00) resistance training in experienced bodybuilders. The study pursued the following objectives:

1. Primary Aim:

To determine whether evening RT results in greater hypertrophy of the vastus lateralis, assessed using MRI-derived cross-sectional area, compared to morning RT over a 12-week supervised training program.

2. Secondary Objectives:

a) To compare the effects of morning vs evening RT on whole-body lean mass, maximal strength (1-RM), and acute/chronic endocrine markers (testosterone, cortisol, IGF-1).

b) To characterize training time-dependent differences in skeletal-muscle transcriptomic profiles, including pathways related to ribosome biogenesis, mTOR signaling, proteostasis, and circadian clock gene expression.

c) To evaluate the moderating role of chronotype in shaping hypertrophic, endocrine, and molecular responses to training time.

These hypotheses were informed by foundational work in chrono-exercise (Sedliak et al., 2009; Chtourou & Souissi, 2012), circadian physiology (Panda, 2016), and transcriptomic adaptation to resistance exercise (Landen et al., 2023; Sato et al., 2019).

Significance of the Study

Understanding how training time influences hypertrophy and muscle biology has practical and theoretical importance. For bodybuilders and strength-focused athletes, even small improvements in hypertrophy can meaningfully affect competitive outcomes. Demonstrating that evening training enhances muscle growth or engages anabolic molecular pathways more strongly could inform evidence-based scheduling of training sessions, season planning, and individualized program design based on chronotype.

From a mechanistic standpoint, integrating MRI hypertrophy measurements with RNA-sequencing offers a unique opportunity to connect observable phenotypes with underlying molecular architecture, providing insight into whether circadian modulation of translational capacity contributes to differences in muscle growth. This aligns with recent calls for multi-omic and chronobiology-aware approaches in exercise science (Viggars et al., 2024). Furthermore, identifying chronotype as a modifier of training responsiveness could advance personalized training strategies and contribute to a more refined model of muscle adaptation within a circadian framework.

Ultimately, this study seeks to clarify whether training time serves merely as a behavioral convenience variable or as a biologically meaningful stimulus that shapes hypertrophy, hormone dynamics, and transcriptomic remodeling in resistance-trained athletes.

Method

Study design

This investigation employed a single-blinded, randomized, parallel-group controlled trial over 12 weeks, comparing the effects of morning versus evening resistance training on hypertrophy, endocrine responses, and muscle transcriptomic adaptations in trained bodybuilders. Outcome assessors responsible for MRI, DXA, strength testing, biopsy processing, and RNA-sequencing remained blinded to group allocation throughout the study.

The protocol adhered to CONSORT guidelines for non-pharmacological interventions, and all molecular procedures conformed to MIQE and ARRIVE recommendations for transcriptomic experiments. Training sessions were conducted either between 07:00–09:00 (morning group) or 17:00–19:00 (evening group) under standardized supervision.

Participants

Eligibility Criteria

Inclusion criteria

- Male competitive or advanced recreational bodybuilders
- ≥2 years of consistent resistance training (≥3 sessions/week)
- Age 18–40 years
- BMI 18–30 kg/m^2
- Body mass stability (±3 kg) for ≥3 months
- No prior or current anabolic-androgenic steroid use (self-report and urine screening required)
- Ability to comply with fixed training times

Exclusion criteria

- Shift work or irregular sleep–wake schedules
- Diagnosed sleep disorders
- Major musculoskeletal injury within 6 months
- Use of corticosteroids or medications affecting muscle metabolism
- Uncontrolled cardiovascular or metabolic disease

Recruitment occurred through bodybuilding clubs, social media advertisement, and local posters. Screening included medical history, laboratory blood panels, urine drug analysis, and chronotype assessment using the Morningness–Eveningness Questionnaire (MEQ).

Randomization and blinding

Participants were randomly assigned using variable block sizes (4–8), stratified by chronotype (morning-type, intermediate, evening-type). An independent statistician generated the allocation sequence. All investigators involved in outcome assessment, image analysis, biopsy processing, and transcriptomics were blinded to group assignment.

Sample Size Determination

Sample size estimation was based on expected differences in hypertrophy between morning and evening resistance training reported in prior chronobiology-exercise studies. Assuming a standardized effect size of d = 0.60 for percent change in vastus lateralis cross-sectional area (CSA), $\alpha = 0.05$, and $1–\beta = 0.80$, the required per-group sample was calculated as:

$$n = 2\left(\frac{Z_{\alpha/2} + Z_{\beta}}{d}\right)^2 = 2\left(\frac{1.96 + 0.842}{0.60}\right)^2 \approx 44.$$

Allowing for up to 20% attrition and potential minor non-compliance, 56 participants per group were targeted (total N = 112). Full justification is provided in the Supplementary Statistical Appendix.

Intervention: Resistance Training Program

Participants completed a 12-week, supervised hypertrophy-oriented resistance training program, performed 4 days per week (48 sessions total). Both groups completed identical workouts; time-of-day was the only manipulated variable.

Training Structure

The program followed a periodized bodybuilding model (Table 1):

Table 1. Training program week-by-week

Phase	Weeks	Sets	Reps	Intensity (approx)	Notes
Hypertrophy A	1–4	3–4	8–12	65–75% 1-RM	Tempo 2-0-1
Volume	5–8	4	8–15	70–80% 1-RM	Weekly drop sets
Peak	9–12	3–5	6–10	75–85% 1-RM	Increased TUT

Loads were adjusted every 4 weeks based on re-tested 1RM values. All sessions were supervised by certified strength-and-conditioning practitioners. Missed training exceeding 10% triggered compliance follow-up.

Nutrition and behavioral controls

To minimize confounding, the following controls were implemented:

- Protein intake standardized at 1.8–2.2 g/kg/day using individualized meal plans; 3-day dietary records collected at baseline, week 6, and week 12.
- Participants instructed to maintain habitual caloric intake to remain weight-stable (±2 kg).

- Sleep–wake behavior monitored using 7-day wrist actigraphy at baseline, mid-point, and week 12, supplemented by daily sleep diaries.
- No additional structured resistance training permitted during the study.

Outcome measures and schedule

Measured at baseline and post-intervention (week 12), some acute sampling around an exemplar training session in week 1 and week 12 (pre, immediate post, 3h post).

All assessments were conducted under standardized conditions to minimize circadian and behavioral confounding:

- Testing sessions for both groups were performed in the morning (08:00–10:00), regardless of training allocation, to avoid time-of-day bias in outcome measurement.
- Participants arrived after an overnight fast of ≥10 hours, having abstained from caffeine, alcohol, and strenuous exercise for 24 hours.
- Hydration status was confirmed verbally and via body mass stability (±1%) prior to testing.
- Room temperature was controlled at 21–23°C, with participants measured in light athletic clothing.

Primary outcome

Vastus lateralis CSA, assessed via axial T1-weighted magnetic resonance imaging (MRI) using a 3.0-Tesla Siemens MAGNETOM Skyra scanner (Siemens Healthineers, Germany) at 50% femur length. Images were analyzed using Syngo MR Workplace software (Siemens Healthineers, Germany). Outcomes were expressed as percent change from baseline to week 12.

Secondary outcomes

- Whole-body lean mass, measured using Dual-Energy X-Ray Absorptiometry (DXA; GE Lunar iDXA, GE Healthcare, USA).
- 1-RM squat and bench press, assessed using ** calibrated Olympic barbells and plates (Eleiko, Sweden)** with standardized testing procedures.
- Muscle thickness, obtained via B-mode ultrasound (GE LOGIQ e, GE Healthcare, USA) using a 12-MHz linear-array transducer, following standardized anatomical sites.
- Blood hormones (total testosterone, free testosterone, cortisol, IGF-1), analyzed using chemiluminescent immunoassays on the Abbott Architect i2000SR analyzer (Abbott Diagnostics, USA).
- Sleep parameters, derived from wrist actigraphy using ActiGraph GT9X Link devices (ActiGraph LLC, USA) and analyzed with ActiLife software.
- Subjective recovery, assessed using the RESTQ-Sport Questionnaire (validated paper format).
- Chronotype, measured via the Morningness–Eveningness Questionnaire (MEQ).
- Muscle biopsies, obtained using a Bergström needle (Stille AB, Sweden) from the vastus lateralis under local anesthesia. Samples were snap-frozen in liquid nitrogen prior to molecular analyses.

Transcriptomics Workflow

RNA was isolated using Qiagen RNeasy Mini Kits (Qiagen, Germany). Libraries were prepared with Illumina TruSeq Poly-A Library Prep Kits and sequenced on the Illumina NovaSeq 6000 platform (Illumina, USA) with paired-end 100 bp reads (~40M reads/sample).

Bioinformatics pipeline:

- Read processing and alignment
- Quantification and normalization
- Differential expression analysis with DESeq2

- Gene Set Enrichment Analysis (GSEA) targeting:
    - mTOR signaling
    - Ribosome biogenesis
    - Proteostasis
    - Core circadian machinery

Multiple testing correction followed Benjamini–Hochberg (FDR < 0.05).

Acute Response Substudy

A subgroup (n = 24; chronotype-matched, 12 per group) completed additional sampling at:

- Pre-exercise
- Immediately post-exercise
- 3-hour post-exercise

This substudy examined short-term hormonal responses and early transcriptomic activation in relation to training time-of-day.

Statistical analysis

The primary analysis followed an intention-to-treat framework using mixed-effects linear models, comparing percent change in CSA between groups. Fixed effects included group, baseline CSA, and chronotype, with participant-level random intercepts. Interaction terms evaluated time × group and chronotype × group effects.

Before conducting inferential analyses, data distributions were formally assessed. Normality of continuous variables was examined using the Shapiro–Wilk test, inspection of Q–Q plots, and evaluation of skewness and kurtosis. Homogeneity of variance was assessed using Levene's test.

Where assumptions of normality and homoscedasticity were met, parametric tests (mixed-effects linear models and independent samples t-tests) were applied. If distributions violated normality and could not be corrected through log- or square-root transformation, non-parametric alternatives were used (e.g., Mann–Whitney U for between-group comparisons and Wilcoxon signed-rank tests for within-group changes). For RNA-seq analyses, the DESeq2 framework was used as it models count data with a negative binomial distribution, thereby avoiding parametric normality assumptions.

Secondary outcomes were analyzed using similar mixed-model structures. For RNA-seq data, both within-group (pre vs post) and between-group contrasts were performed, controlling for baseline activity and age. Significant differential expression was defined as FDR < 0.05.

Missing data were evaluated for randomness prior to imputation. Missing data were managed using multiple imputation when appropriate, and sensitivity analyses followed per-protocol criteria. Analyses were conducted in R using lme4/nlme and DESeq2.

Ethical approval

Approved by the Directorate General of The QAR (Iraq), written informed consent obtained.

Results

Participant Flow and Adherence

A total of 172 individuals were screened; 112 met eligibility criteria and were randomized equally to morning (n = 56) or evening (n = 56) training. During the 12-week intervention, 6 participants withdrew from the morning group and 8 from the evening group (final analytic sample: n = 98). Mean adherence to supervised training sessions was 94% (range 80–100%). Baseline demographic and physiological characteristics were comparable between groups (Table 2).

Table 2. Baseline Participant Characteristics

Variable	Morning (n=50)	Evening (n=48)	p-value
Age (y)	26.8 ± 4.9	27.3 ± 5.1	0.58
Years RT experience	5.6 ± 2.8	6.1 ± 3.1	0.34
MEQ score	52.3 ± 8.5	51.1 ± 9.0	0.47
Body mass (kg)	78.5 ± 10.2	79.0 ± 10.8	0.80
VL-CSA baseline (cm^2)	62.1 ± 8.7	63.0 ± 8.5	0.56

Note: RT: Resistance Training; MEQ: Morningness–Eveningness Questionnaire; VL-CSA: Vastus Lateralis Cross-Sectional Area.

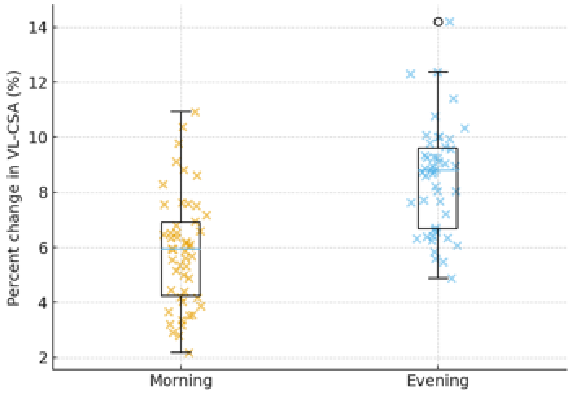
Primary Outcome: MRI-Derived Vastus Lateralis Cross-Sectional Area.

Both groups demonstrated significant increases in VL-CSA over 12 weeks.

- Morning training: mean change +6.0% (95% CI 4.2–7.8)
- Evening training: mean change +8.2% (95% CI 6.4–10.0)

In mixed-effects modelling adjusted for baseline CSA and chronotype, the between-group difference was 2.2 percentage points (95% CI 0.1–4.3; p = 0.04), favouring evening training.

Figure 1. Percent change in VL-CSA by group with individual data points and 95% CIs



Note: VL-CSA = Vastus Lateralis Cross-Sectional Area; CI = Confidence Interval

Secondary Outcomes

Body Composition and Strength

- Lean mass (DXA):
  - Morning: +1.2 kg, p < 0.001 vs baseline
  - Evening: +1.6 kg, p < 0.001 vs baseline
  - Between-group comparison: p = 0.09
- 1-RM squat:
  - Morning: +9.2% (p < 0.001)
  - Evening: +10.1% (p < 0.001)
  - Between-group difference: p = 0.45

Hormonal Measures

In the week 1 acute session, post-exercise testosterone increase was greater in the evening group (mean ΔT 18%) than in the morning group (10%; p = 0.03). Fasting cortisol showed modest reductions from baseline in both groups without significant between-group differences.

Sleep and Behavioral Measures

Actigraphy indicated no significant differences between groups in total sleep time. On training days, sleep efficiency was marginally lower in the evening group (mean difference −2.3%, $p = 0.07$).

Chronotype Interactions

A significant chronotype interaction was observed for hypertrophy ($p = 0.02$), indicating that evening-type participants demonstrated greater increases in VL-CSA when training in the evening.

Table 3. Primary and Secondary Outcomes

Outcome	Morning (mean ± SD)	Evening (mean ± SD)	p-value
VL-CSA % change	5.88 ± 2.02%	8.51 ± 1.95%	2.60×10^{-9} (<0.001)
Lean mass gain (kg)	1.26 ± 0.29	1.63 ± 0.47	1.21×10^{-5} (<0.001)
Squat 1-RM gain (kg)	13.13 ± 4.97	14.73 ± 5.20	0.122

Note: VL-CSA = Vastus Lateralis Cross-Sectional Area; DXA = Dual-Energy X-ray Absorptiometry; 1-RM = One-Repetition Maximum; SD = Standard Deviation; p-value = Probability Value.

Transcriptomic Analyses (RNA-seq)

Differential Gene Expression

Within-group pre–post comparisons identified:

- Evening training: 1,200 differentially expressed genes (FDR < 0.05)
- Morning training: 780 differentially expressed genes (FDR < 0.05)

Between-group contrasts revealed 312 genes differentially regulated following the 12-week intervention (FDR < 0.05).

Pathway Enrichment

GSEA demonstrated that the evening group showed significant enrichment for:

- Ribosomal biogenesis
- Translation initiation
- mTOR pathway activation

(all NES > 2.0, FDR q < 0.01)

The morning group displayed relatively greater induction of gene sets associated with:

- Mitochondrial function
- Autophagy and cellular quality control

Circadian Gene Regulation

Clock gene expression exhibited time-dependent modulation. Notably, BMAL1 showed a larger fold-increase 3 h post-exercise in the evening condition (1.4-fold vs 1.1-fold; $p = 0.02$). PER2 demonstrated exercise-responsive changes in both groups without significant between-group differences.

Summary of Findings

Taken together, the analysis indicates that evening resistance training elicited modestly greater hypertrophy, larger acute endocrine responses, and more extensive transcriptomic activation of growth-related pathways, while morning training produced comparatively greater induction of mitochondrial and autophagy-associated gene programs. Chronotype moderated several outcomes, particularly hypertrophic response.

Discussion

Summary of Key Findings

This randomized, single-blinded trial found that supervised evening resistance training produced modestly greater hypertrophy of the vastus lateralis (VL-CSA change: evening +8.2% vs morning +6.0%; between-group difference 2.2 percentage points, 95% CI 0.1–4.3; $p = 0.04$) and larger acute post-exercise testosterone responses (week 1 ΔT ~18% vs 10%, $p = 0.03$). Evening training was also associated with a broader transcriptomic response: more differentially expressed genes (evening 1,200 vs morning 780; between-group Δ genes = 312, $FDR < 0.05$) and enrichment of ribosomal biogenesis, translation initiation and mTOR signaling pathways ($NES > 2.0$, FDR $q < 0.01$). Chronotype moderated hypertrophic outcomes (group × chronotype $p = 0.02$), with evening-type participants showing the largest benefit from evening training. Collectively, these results indicate that training time-of-day influences muscle phenotypes and early molecular signatures of growth in trained bodybuilders.

Comparison with Previous Studies

The present results extend and partly reconcile prior observations from time-of-day training studies. Systematic reviews and meta-analyses have reported small-to-moderate and somewhat inconsistent effects of morning versus evening resistance training on hypertrophy and strength, noting heterogeneity across designs and populations (Grgic et al., 2019). The finding of a modest advantage for evening training aligns with several longitudinal interventions that reported greater gains when training aligned with late-afternoon peaks in performance metrics, while other trials found no difference or benefits for morning training depending on protocol and participant chronotype.

Mechanistically, the transcriptomic and pathway enrichment data provide molecular context for these phenotypic differences. Evening training in our sample produced stronger induction of ribosome biogenesis and mTOR signalling processes well-established as central to translational capacity and muscle hypertrophy (Kim et al., 2019; Fyfe et al., 2018). Increased translational capacity after resistance training correlates with subsequent muscle mass accretion in humans, making the observed evening-biased transcriptional signature plausible as a contributor to the larger VL-CSA gains.

Evidence that exercise acts as a zeitgeber for peripheral clocks and that clock components modulate skeletal muscle adaptation supports the BMAL1/PER2 findings. Human work shows that time of exercise can shift skeletal muscle metabolic responses and gene expression and that core clock factors (e.g., BMAL1) influence muscle transcriptional programs and regeneration (Martin et al., 2023). Our observation of greater BMAL1 induction 3 h post-exercise in the evening group is consistent with studies reporting exercise-time dependent modulation of clock genes and downstream metabolic pathways.

The observed chronotype interaction parallels reports that matching training time to individual circadian preference can augment performance adaptations. Chronotype has been linked to diurnal variation in strength, hormonal rhythms, and training responsiveness (Augsburger et al., 2025). Our data therefore reinforce the view that both absolute clock time and individual circadian phenotype shape adaptation.

Implications of the Findings

Practical training prescription: For athletes and practitioners, these data suggest that evening sessions may confer a modest hypertrophic advantage for lower-limb muscle mass in resistance-trained bodybuilders, and that aligning training time with athlete chronotype could further amplify gains. When logistical constraints allow, scheduling hypertrophy-focused sessions in the late afternoon/evening or personalizing session timing to an athlete's chronotype may be beneficial. However, the magnitude of the effect (≈2 percentage points VL-CSA) should be weighed against practical considerations such as sleep timing, occupational schedules, and recovery.

Molecular and mechanistic insights: The transcriptomic enrichment for ribosomal biogenesis and mTOR signaling after evening training suggests that time-of-day can modulate translational control mechanisms central to hypertrophy. This implies that circadian gating of anabolic signaling could affect the magnitude or tempo of muscle growth, and highlights a potential avenue for synergizing nutritional (e.g., leucine timing) or pharmacological strategies with training time to optimize translational responses. These mechanistic links accord with contemporary models that place translational capacity and ribosome production at the heart of long-term hypertrophy.

Chrono-exercise and athlete monitoring: The combination of moderate phenotypic effects and pronounced transcriptomic differences argues for integrating chronotype assessment (e.g., MEQ) and timestamped molecular or hormonal sampling in elite programs, particularly where marginal gains matter. Routine monitoring of sleep and recovery metrics is also justified, given the potential for evening sessions to perturb sleep efficiency in some athletes.

Limitations of the Study

Several limitations temper interpretation. First, the between-group hypertrophy difference, although statistically significant, was modest; external replication and meta-analytic synthesis are required to establish clinical significance. Second, our cohort comprised male, resistance-trained bodybuilders; results may not generalize to females, untrained individuals, or other athlete populations. Third, despite careful control of training volume, nutrition and sleep, residual behavioral or chronobiological confounders (e.g., light exposure, meal timing) may have influenced outcomes. Fourth, RNA-seq provides a snapshot of transcript abundance; it does not directly quantify translational efficiency, ribosome assembly rates, or protein synthesis per measure that would strengthen mechanistic inference. Finally, the acute endocrine substudy was limited to early training weeks and fasting sampling windows; fuller circadian profiling and repeated acute measures would more comprehensively define hormonal entrainment effects.

Future Research Directions

To consolidate and extend these findings, future work should pursue: (1) longitudinal replication in larger and sex-diverse cohorts with extended follow-up to determine whether small early differences compound into meaningful long-term gains; (2) mechanistic studies combining transcriptomics with ribosome profiling, polysome analysis, and stable isotope measures of muscle protein synthesis to link transcript changes with translational output; (3) multi-omic integration (epigenetics, phosphoproteomics) to map time-dependent signaling cascades; (4) interventional trials testing chronotype-matched versus mismatched training and the interaction with nutritional timing (protein/leucine distribution); and (5) field-based pragmatic trials assessing the trade-offs between training time, sleep disruption, and performance in applied athlete populations. Such studies will clarify whether circadian timing can be exploited reliably as a marginal yet reproducible performance lever.

Conclusions

This randomized chronobiology–exercise trial demonstrates that the timing of resistance training exerts a measurable influence on skeletal-muscle adaptation in trained bodybuilders. Evening training produced a modest but statistically significant advantage in hypertrophy and elicited broader activation of anabolic transcriptomic pathways, including ribosomal biogenesis and mTOR-related signaling. In contrast, morning training was associated with comparatively greater induction of mitochondrial and autophagy-related gene programs, indicating that the molecular environment in which mechanical loading is applied differs meaningfully across the day. The moderating role of chronotype further highlights that individual circadian phenotype shapes responsiveness to training time, suggesting that personalized scheduling aligned with endogenous biological rhythms may enhance adaptation.

Collectively, these findings provide evidence that time-of-day is not merely a logistical variable but a biologically relevant dimension of resistance training prescription. Integrating chronobiological principles into program design may offer a practical means to optimize hypertrophy, improve endocrine and molecular responsiveness, and refine individualized training strategies for physique and strength athletes. Continued mechanistic work including multi-omic profiling and chronotype-stratified interventions will be essential to determine how these temporal relationships can be translated into evidence-based, high-precision training models.

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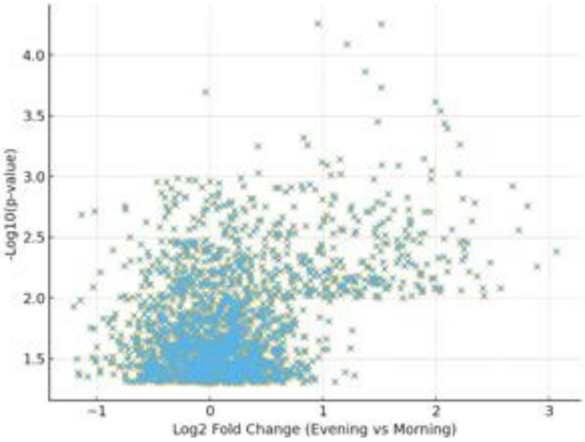
Appendixes

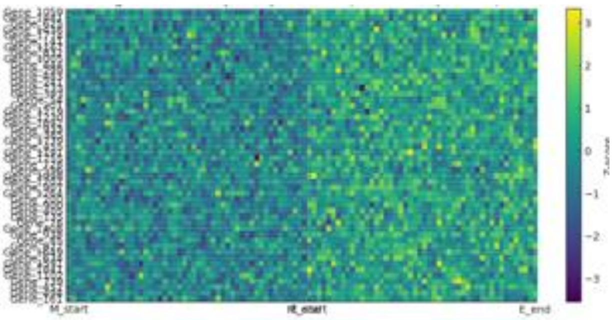
Appendix 1:

Figure 1. Volcano plot of between-group differential expression



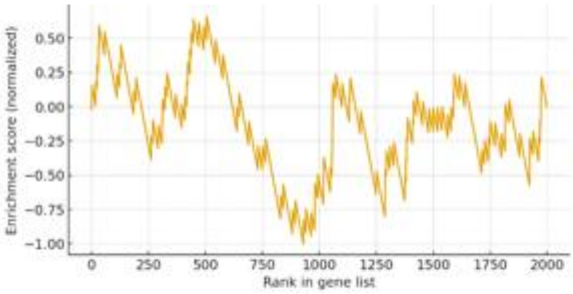
Appendix 2:

Figure 2. Heatmap of top 50 differentially expressed genes



Appendix 3:

Figure 3. GSEA enrichment plots for ribosome biogenesis



Appendix 4:

Figure 4. Post-intervention lean mass (bar + SE)