RESEARCH ARTICLE

Chrono-Resistance Training Optimizes Hippocampal Oxidative Balance, Reduces Visceral Fat, and Enhances Memory in Healthy Rats Model: Preliminary Findings

Nur Aisyah Anuar[1,2], Norwahidah Abdul Karim[3], Nurul Hazlini Suhaida Mohd Nazri[2], Muhamad Halil Fikri Laililnizan[2], Farah Wahida Ibrahim[2,4], Muhammad Hafiz Zuhdi Fairof[1,2], Amirul Hafiz Ahmad Abdullah[2,4], Daren Kumar Joseph[1], Hameed Ali Al-Saraf[5], Nor Fadilah Rajab[1,2], Arimi Fitri Mat Ludin[1,*]

[1]Centre for Healthy Ageing and Wellness (HCARE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jl Raja Muda Abd Aziz, Kuala Lumpur 53000, Malaysia
[2]Biomedical Science Programme, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jl Raja Muda Abd Aziz, Kuala Lumpur 53000, Malaysia
[3]Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia, Universiti Kebangsaan Malaysia, Jl Raja Muda Abd Aziz, Kuala Lumpur 53000, Malaysia
[4]Center for Toxicology and Health Risk Studies (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jl Raja Muda Abd Aziz, Kuala Lumpur 53000, Malaysia
[5]Department of Physiology, Faculty of Medicine, Kuwait University, Safat, Kuwait City 13110, Kuwait

*Corresponding author. Email: arimifitri@ukm.edu.my

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Abstract

BACKGROUND: Exercise acts as a non-photic zeitgeber and reduces visceral adiposity, a key source of oxidative stress that impairs hippocampal integrity and cognitive function. Excess visceral fat promotes systemic redox imbalance, compromising hippocampal-dependent spatial memory. Early oxidative disturbances preceded overt pathology and may contribute to neurodegenerative risk, and exercise may alleviate its risk. However, the metabolic and cognitive outcomes are rarely assessed together in resistance exercise models. Therefore, this study investigated chrono-resistance training effects on visceral fat, hippocampal oxidative status, and spatial memory in healthy rats.

METHODS: Wistar rats were assigned to Early-active Control (EC), Early-active Exercise (EE), Late-active Control (LC), and Late-active Exercise (LE). Exercise was performed three times per week for 8 weeks along a 180-cm ladder-climbing track. Before and after the exercise protocol, the rats' grip strength, visceral fat mass, and spatial memory (Y-maze) were assessed. Hippocampal oxidative markers, including malondialdehyde (MDA) and glutathione (GSH) were also measured with thiobarbituric-acid-reactive-substances (TBARS) assay and modified Ellman method, respectively.

RESULTS: At week-8, LC showed greater grip strength than EC (p=0.002), while EE maintained higher strength than EC (p<0.001). LC reduced visceral fat compared with EC (p<0.001), with further reductions in EE (p<0.001). Hippocampal MDA was lower in EE and LC than LE (p≤0.002), whereas GSH was highest in EE (p<0.001). Spatial memory declined in EC (p=0.004) but improved in LC (p=0.015), with LC outperforming EC (p<0.001).

CONCLUSION: Rats assigned to EE chrono-resistance training shows better results in improving grip strength, adiposity, and redox balance, whereas LC chrono-resistance training shows better results in preserving spatial working memory. This suggests that chrono-resistance training improves metabolic and hippocampal redox outcomes and modulates memory, indicating that exercise timing shapes metabolic–brain adaptations.

KEYWORDS: chrono-exercise, grip strength, hippocampus, memory cognition, oxidative stress, visceral fat

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Introduction

Exercise is a prominent external non-photic zeitgebers as evidence in preclinical study following voluntary exercise.(1) Circadian rhythms regulate oxidative stress dynamics in the hippocampus, where robust 24-hour oscillations in antioxidant defenses and oxidative damage markers underscore its chrono-dependent and metabolically vulnerable nature.(2) Such vulnerability is functionally important, as the hippocampus supports spatial memory, a core domain of cognitive function required for efficient learning and adaptive behavior.(3) Misalignment of circadian rhythms can impair metabolic homeostasis, elevate oxidative stress, and promote obesity and related metabolic disorders.(4) Notably, visceral adiposity represents a key metabolic source of systemic oxidative stress that can compromise hippocampal integrity and associated cognitive processes, as evidenced in young adults showing strong positive correlations between visceral fat and circulating cellular stress metabolites.(5) These findings highlight an interconnected pathway linking circadian regulation, adiposity, and hippocampal-dependent function, which may be favorably modulated by exercise.

Aerobic exercise has been widely used as a model to investigate brain performance.(6) Fewer studies have examined resistance exercise concerning this area. However, narrative reviews and clinical trials suggests that resistance exercise can also support cognitive health, particularly in aged population.(7) Reviews on physical exercise indicate that both endurance and resistance exercise can perturb cellular redox homeostasis and stimulate antioxidant signaling pathways, enhancing cellular defenses over time.(8) This suggests that resistance exercise has the potential to induce adaptive redox responses, though evidence in the healthy young brain remains limited. All of these findings highlight the aligning physiological stimuli with favorable circadian phases can enhance endogenous antioxidant capacity and manage redox homeostasis. Circadian preference of oxidative stress thus represents a key regulatory whereby redox modulation potentially preceding observable functional improvement.(9)

Resistance exercise promotes interconnected improvements in muscular performance, metabolic health, and hippocampal-dependent cognition through linked peripheral and central adaptations. Experimental evidence demonstrates that eight weeks of ladder climbing resistance exercise in high fat diet mice shown to enhanced grip strength that is inversely correlated with fat mass.(10) Improved functional performance is associated with better metabolic regulation, including reductions in visceral adiposity.(11) The reduction of visceral fat is especially helpful since it is metabolically active and secretes pro-inflammatory cytokines that promote reactive oxygen species (ROS) generation.(12) Consistently, clinical comparisons across adipose depots indicate that visceral fat shows the strongest negative association with cognitive performance.(13) A core component of cognitive function include memory is critical for learning and adaptive behavior. This domain is highly dependent on hippocampal integrity, which is particularly vulnerable to oxidative stress.(14) Beyond metabolic outcomes, resistance exercise may also benefit central nervous system function. For example, resistance training in aged rats has been shown to improve learning and memory while promoting hippocampal adaptations consistent with enhanced neuroplasticity.(15)

Despite these observations, the chrono-dependent effects of resistance exercise on metabolic and hippocampal oxidative adaptations remain poorly understood. This study therefore aimed to investigate the effects of chrono-resistance training on visceral adiposity, hippocampal oxidative balance, and memory in healthy rats. Using a healthy rat model allows the observation of natural circadian oscillations in hippocampal oxidative status and systemic metabolism without confounding influences of disease or aging, thereby providing a baseline for understanding how chrono-exercise may optimize metabolic and cognitive health.

Methods

Experimental Design and Animal Grouping

Thirty-six male Wistar rats aged 8-10 weeks old and with initial body weight of 267.44±23.98 g were employed in this study. Sample size was determined a priori using the resource equation method appropriate for animal studies, calculated using ANOVA-based formula.(16) Animals were monitored for distress with predefined humane endpoints and housed under controlled conditions (~24 °C, 12 h light–dark cycle, zeitgebers time (ZT)0–ZT12, 07:00–19:00 h) with *ad libitum* food and water. Rats were randomly assigned (n=9) to Early-active Control (EC), Early-active Exercise (EE), Late-active Control (LC), and Late-active Exercise (LE) groups, stratified by body weight to balance distribution, and housed in pairs. Body weight was recorded weekly throughout the experimental period (W0–W8). After one week of habituation and one week of track familiarization,

baseline assessments were conducted, including grip strength (W0; ZT2–ZT9) and cognitive performance (W0; ZT14–ZT17). Exercise and control sessions were conducted simultaneously according to their respective chrono-exercise, with consistent order of animal handling maintained across all sessions. Grip strength was reassessed at week-4 and -8, while cognitive performance was repeated at week-8 only to minimize learning effects and both was conducted at the same ZT. Soleus, gastrocnemius and visceral adiposity and hippocampal tissues were collected at week-8 (Figure 1).

Resistance Exercise Protocol

Exercise equipment was recreated, adapting the 130 cm total length.(17) Animal were familiarized with the exercise track for a week. EE and EC groups exercised during the early active phase (ZT13), while LE and LC trained during the late active phase (ZT1).(18) ZT13 and ZT1 represent active-phase onset and rest-phase latency respectively, providing a clinical translational framework for chrono-exercise timing. ZT13 (20:00 h) marks the onset of the rodent active phase, characterised by increased locomotor activity and core body temperature, reflecting physiological readiness.(19) In contrast, ZT1 (08:00 h) corresponds to the rest phase, with reduced behavioural activity and circadian drive where increased sensitivity to non-photic stimuli such as exercise. (20,21) Sessions involved 14–16 repetitions with ≤30 s rest, three non-consecutive days per week for 8 weeks, with progressive load increments every two weeks (25%, 50%, 75%, 100% of body weight).(22) All exercise and sham sessions were conducted under identical environmental conditions. Controls performed sham exercise at a constant minimal load (25% body weight) (23) to provide baseline exposure to the apparatus and handling, isolating the effects of progressive loading. Minimal tactile stimulation was used to encourage participation. No aversive stimuli (*e.g.*, electrical shock or forced pacing) were applied, and animals performed the task voluntarily after habituation, minimizing stress-related confounding.

Analysis of Cognitive Performance

Spatial memory was assessed using the 8-min Y-maze (24), with spontaneous alternation (%) reflecting hippocampal-dependent working memory. The apparatus consisted of three identical arms positioned at 120°. Rats were placed at the end of one arm and allowed to freely explore. Arm entry was defined as all four limbs entering an arm, and sequences were recorded. Spontaneous alternation was

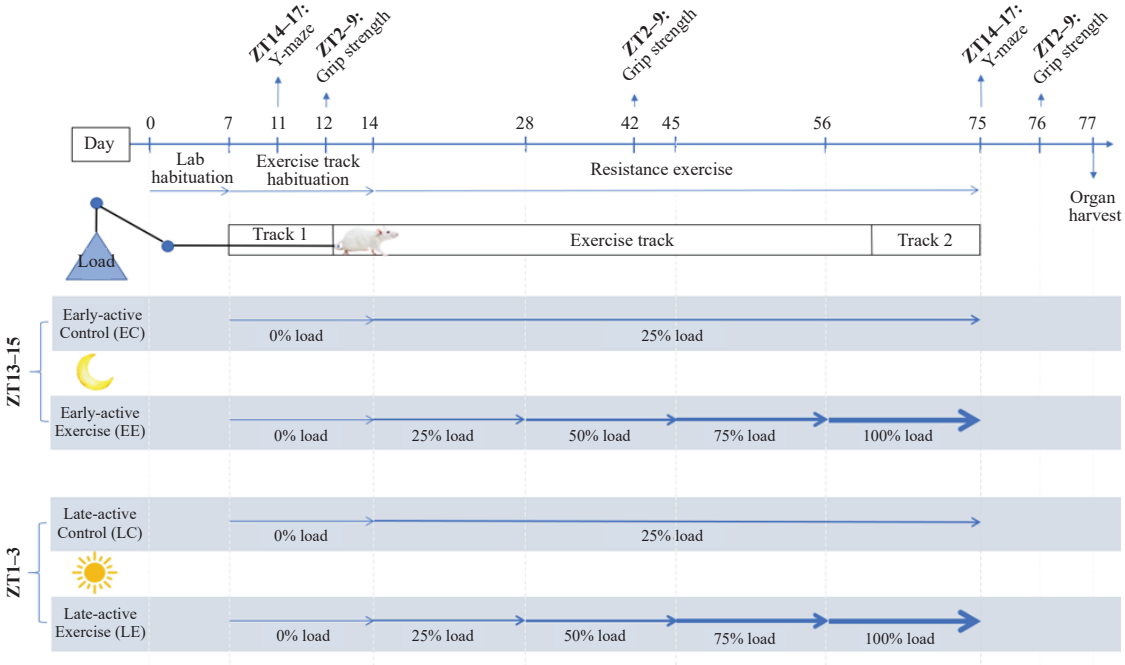

Figure 1. Experimental design of current study. Following 1 week of laboratory habituation and 1 week of exercise track familiarization, baseline grip strength (ZT2–ZT9) and Y-maze (ZT14–ZT17) were measured. Thirty-six rats were randomly assigned (n=9/group) into Early-active Control (EC), Early-active Exercise (EE), Late-active Control (LC), and Late-active Exercise (LE). Exercise groups (EE, LE) underwent progressive resistance training (load: 0–100% body weight, increment every 2 weeks) three times weekly, while control groups (EC, LC) performed sham exercise at constant minimal load (25%). Early-active groups trained at ZT13 (active-phase onset), whereas late-active groups trained at ZT1 (rest-phase period). Grip strength was reassessed at weeks 4 and 8, and Y-maze at week 8. All animals were euthanized at week 8 for tissue collection.

defined as consecutive entries into three different arms (*e.g.*, ABC, BCA). The percentage alternation was calculated as: (alternations / [total entries – 2]) × 100.

Analysis of Skeletal Muscle Performance

Grip strength reflecting skeletal muscle force and neuromuscular coordination mainly via neural adaptation was measured to assess muscle performance. Using PowerLab ADInstruments (ADInstruments, Sydney, Australia) and handling adapted from previous study.(25) Three readings per rat (g) were averaged.

Visceral Adiposity and Hippocampal Tissue Collection

Following completion of the experimental protocol, animals were euthanized under CO_2 exposure in a chamber. Visceral fat and brain tissues were rapidly dissected. Visceral adiposity measurement reflects lipid metabolism and systemic metabolic health, as abdominal visceral adiposity contributes to oxidative imbalance. Abdominal visceral fat was surgically isolated from the pancreas, retroperitoneal, mesenteric, and gonadal regions (26), then weighted.

Brain was rapidly harvested, washed with cold saline (4°C), and kept on ice. Hippocampal regions were isolated. The tissues were immediately stored at –80°C until further analysis. Prior to analysis, hippocampal tissue samples were homogenized in 1.15% potassium chloride (KCl; Sigma, St. Louis, MO, USA) at a ratio of 10 mL/g (v/w) using an Ultra-Turrax T25 homogenizer (IKA, Staufen, Germany). Homogenates were centrifuged at 4000 rpm for 20 min at 4°C using a Hettich Zentrifugen centrifuge (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany), and the resulting supernatants were collected for subsequent biochemical analyses. All homogenization and centrifugation steps were performed on ice or at 4°C to minimize oxidative degradation of tissue components.

Protein Determination

The protein content of tissue homogenates was determined using the Lowry method. Briefly, 0.1 mL of sample or bovine serum albumin (BSA, 1 mg/mL; Sigma) standard solution was mixed with 1 mL of Lowry working reagent, prepared by combining 0.5 mL of 2% sodium potassium tartrate (Fisher Scientific, Schwerte, Germany), 0.5 mL of 1% copper sulfate (Merck, Darmstadt, Germany), and 49 mL of 2% sodium carbonate in 0.1 M NaOH (Chemiz, Selangor, Malaysia). The mixture was incubated in the dark at room temperature for 30 min, followed by the addition of 0.1 mL Folin–Ciocalteu reagent (1:1; R&M, Hampshire, UK), and incubated for an additional 30 min in the dark. Absorbance was measured at 600 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA), and protein concentrations were determined against a BSA standard curve.

Malondialdehyde (MDA) Level Measurement

Lipid peroxidation was assessed by measuring MDA levels using the thiobarbituric acid reactive substances (TBARS) method (27), reflective of oxidative damage. Fourty nmol/L 1,1,3,3-tetraethoxypropane (TEP; Sigma) stock solution was prepared and used to generate the standard curve. Fifty µL tissue homogenates were mixed with 2.5 mL of 19.93% trichloroacetic acid in 0.06 M hydrochloric acid (TCA/HCl; Chemiz) and incubated at room temperature for 15 min. Subsequently, 150 µL of 1% thiobarbituric acid in 0.05 M NaOH (Chemiz) was added, and the mixture was heated in a water bath at 100°C for 30 min while covered with aluminum foil to prevent evaporation. Once the samples reached room temperature, absorbance was read at 532 nm with a microplate reader. A calibration curve was referred to quantified MDA level and expressed relative to protein concentration.

Reduced Glutathione (GSH) Level Measurement

Reduced glutathione (GSH) levels were measured using a modified Ellman method (28), representing endogenous antioxidant capacity. Ten mM GSH stock solution (Sigma) was prepared in pH 6.5 buffer, and 4 mg/mL 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma) solution was prepared in pH 8.0 buffer immediately before use. Fifty µL tissue homogenates were combined with 40 µL of pH 8.0 buffer and 10 µL DTNB in microplate wells and incubated in the dark at room temperature for 15 min. The absorbance of the yellow-colored 5-thio-2-nitrobenzoic acid product was measured at 412 nm. GSH concentrations were calculated from the standard curve and normalized to protein content of the respective samples.

Statistical Analysis

All analyses were performed using IBM SPSS Statistics 30 (IBM Corporation, Armonk NY, USA), and graphs were generated using GraphPad Prism 9 (GraphPad Software, Boston, CA, USA). Data are presented as mean±SEM. Normality was assessed using the Shapiro–Wilk test. Weekly body weight and Y-maze performance (W0 vs W8) were analyzed using mixed-design ANOVA. Grip strength was analyzed using mixed-design ANCOVA with baseline body weight and baseline grip strength as covariates. Visceral adiposity was analyzed using two-way ANCOVA with

pre-decapitation body weight as a covariate. Hippocampal oxidative markers (MDA and GSH) were analyzed using two-way ANOVA. Bonferroni correction was used for post-hoc comparisons. Statistical significance was set at $p<0.05$.

Results

Comparable Body Weight Gain Across Groups

All 36 rats successfully completed the 8-week exercise protocol with zero dropout. By the end of the exercise period, the intervention groups carried loads equivalent to 100% of their body weight (approximately 400–500 g). There was a significant main effect of time on body weight ($F(9,288)=540.541$, $p<0.001$, $\eta^2p=0.944$), indicating a robust change in body weight across the study period. As illustrated in Figure 2A, all group show a significant week-to-week gain across habituation phase into exercise phase ($p<0.05$) at least until W2 for late-active group and W3 for early-active groups. Afterward, weekly body weight changes were largely non-significant and lacked temporal consistency. In contrast, no significant main effect of group was observed ($F(3,32)=0.285$, $p=0.836$, $\eta^2p=0.026$), indicating comparable overall body weight among groups. Additionally, the group × time interaction was not significant ($F(27,288)=0.423$, $p=0.995$, $\eta^2p=0.038$), demonstrating that the pattern of body-weight change over time did not differ between groups.

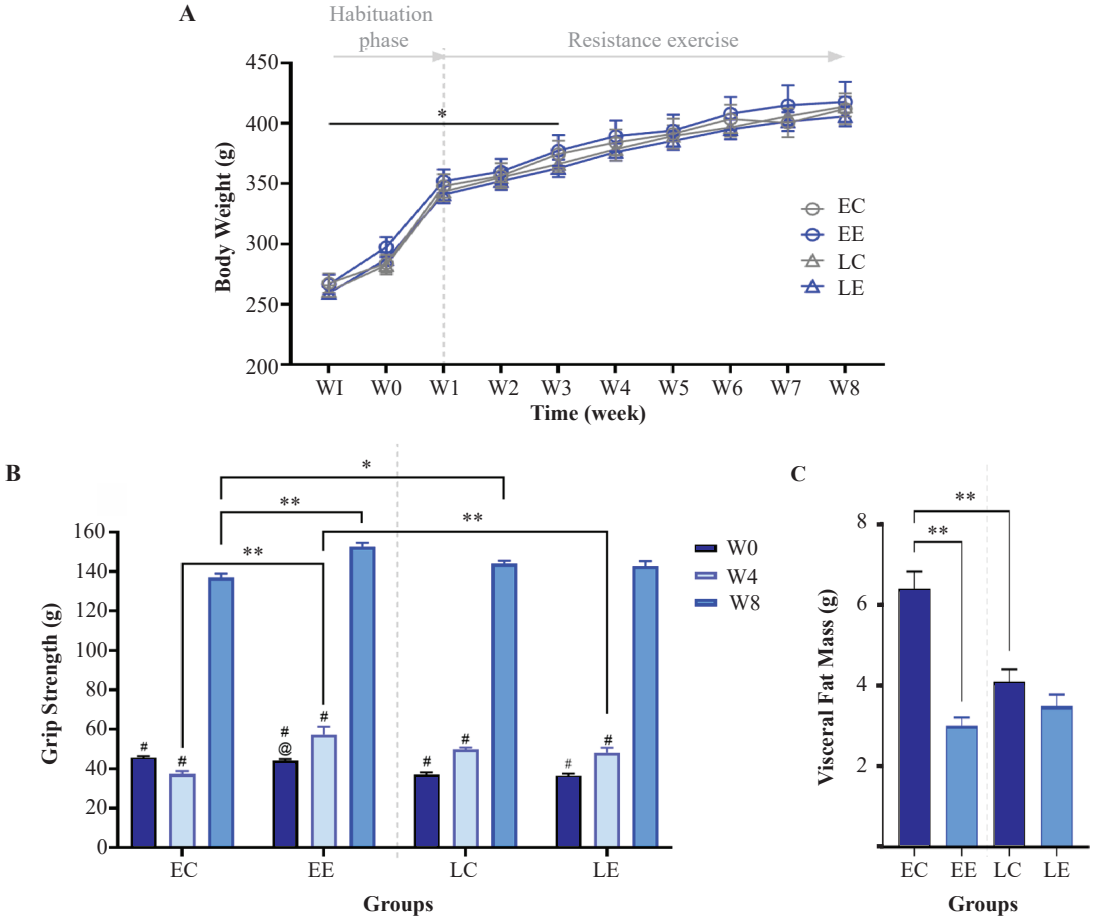

Figure 2. Body composition and muscle performance. A: Weekly body weight (g); Body weight was monitored over 10 weeks (2-week habituation followed by an 8-week intervention) in male Wistar rats assigned to four groups (n=9). Weekly body weight was analyzed using mixed-design ANOVA to assess overall temporal trends and group comparability, rather than as a primary outcome. B: Muscle performance (grip strength, g); All-limb grip strength was assessed at baseline (W0), week-4 (W4), and week-8 (W8) to evaluate neuromuscular performance and validate the resistance exercise protocol (n=6 for LE; n=7 for EC, LC, and EE groups). A mixed-design repeated-measures ANCOVA was conducted with baseline body weight and baseline grip strength included as covariates. C: Visceral adiposity (g); Abdominal visceral fats were collected from pancreatic, retroperitoneal, mesenteric, and gonadal depots after 8 weeks of resistance exercise. Visceral adiposity was analyzed via two-way univariate ANCOVA, with bodyweight prior to decapitation as covariate. (n=4 for LE; n=5 for EC, LC, and EE groups). Greenhouse–Geisser corrections were applied when sphericity was violated, and Bonferroni-adjusted post-hoc comparisons were used. Data are presented as mean±SEM. $^{@}p<0.001$ *vs* respective W4; $^{\#}p<0.001$ *vs* respective W8; *$p<0.05$; **$p<0.001$.

Early-active Exercise Enhances Grip Strength

Grip strength (Figure 2B) was significantly influenced by baseline strength (Time × baseline score: F=3.946, p=0.027, η^2p=0.165), while body weight showed a non-significant trend (p=0.065). A significant time × group interaction was observed (F(2,42)=8.010, p=0.004, η^2p=0.276), alongside time × chrono effect (p=0.050). A robust time × chrono × group interaction (F(2,42)=11.410, p<0.001, η^2p=0.352) indicated chrono-dependent exercise adaptations. At week-4, EP showed higher grip strength than LE (p<0.001). At week-8, EC exhibited lower grip strength than LC (p=0.002). Within the early phase groups, EE demonstrated greater strength than EC at both week-4 and -8 (both p<0.001). The EE group exhibited progressive increases across all time points (week-0 < week-4 < week-8, all p<0.001). In EC, LC, and LE groups, significant increases were primarily observed between baseline (W0) and week-8 (p<0.001), with exponential week-4 to -8 improvements (p<0.001).

Early-active Exercise Exhibits Lower Visceral Adiposity

Visceral adiposity (Figure 2C) analysis of covariance with body weight as a covariate (Group × Body weight) showed no significant interaction, F(1,12)=0.082, p=0.780, η^2p=0.007. Main effects showed a significant group effect on visceral fat (F(1,14)=16.291, p=0.001, η^2p=0.538), with post-hoc comparisons indicating higher visceral fat in EC than LC (p<0.001) and EC than EE (p<0.001).

Early-active Exercise Confers Lower Oxidative Damage

Oxidative damage (Figure 3A), reflected by MDA levels, was analyzed for group and chrono effects. A significant interaction was observed (Group × Chrono: F(1,30)=14.517, p<0.001, η^2p=0.326). Post-hoc comparisons showed that MDA was lower in the EE group compared with LE (p=0.002) and lower in LC compared with LE (p=0.001).

Early-active Exercise Showed Superior Antioxidant Level

Antioxidant status (Figure 3B), reflected by GSH levels, showed a significant interaction between chrono and group (Chrono × Group: F(1,28)=94.790, p<0.001, η^2p=0.772). Post-hoc analyses indicated higher GSH in EE than LE (p<0.001), lower GSH in EC than EE (p<0.001), and lower GSH in LC than LE (p<0.001).

Chrono-exercise Influences Longitudinal Changes in Spatial Working Memory

Spatial working memory (Figure 4) was assessed using spontaneous alternation in the Y-maze. No significant interaction was found between time and load (Time × Group: F(1,15)=0.135, p=0.719, η^2p=0.009). However, a significant Time × Chrono interaction was observed (F(1,15)=18.837, p<0.001, η^2p=0.557), while the three-way interaction (Time × Chrono × Group) was not significant (F(1,15)=3.638, p=0.076, η^2p=0.195). Post-hoc analysis showed reduced performance in the EC group from baseline to week-8 (week-0 > week-8, p=0.004), whereas the LC group improved over time (week-0 < week-8, p=0.015). At week 8, LC demonstrated higher spatial memory performance than EC (p<0.001).

Discussion

This study to provide preliminary findings on whether chrono-exercise differentially influence these outcomes in healthy animals, with all parameters measured presented in Table 1. By the end of the exercise period, the intervention groups carried loads equivalent to 100% of their body weight (approximately 400–500 g). Across all groups, body weight increased over time but in temporal pattern.

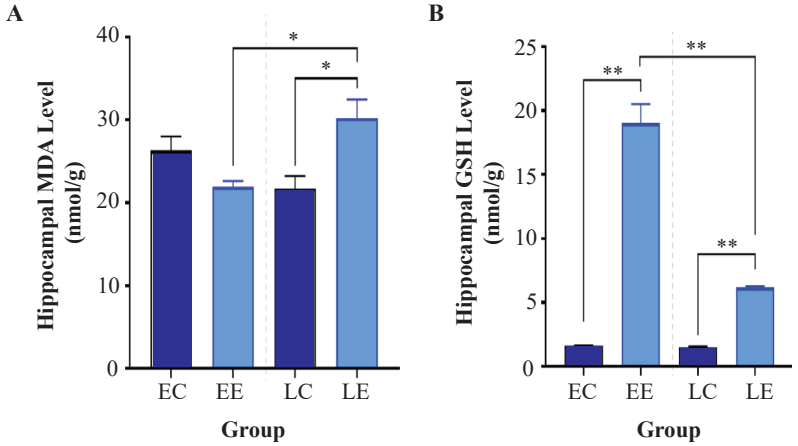

Figure 3. Hippocampal oxidative balance. A: Hippocampal MDA (nmol/mg protein); measured after 8 weeks of intervention (n=8 for EE and LC; n=9 for EC and LE). B: Hippocampal GSH (nmol/mg protein); measured after 8 weeks of intervention (n=7 for EE; n=8 for EC and LE; n=9 for LC). Both parameters were analyzed via two-way univariate ANOVA; Greenhouse–Geisser corrections were applied when sphericity was violated, and Bonferroni-adjusted post-hoc comparisons were used. Data are presented as mean±SEM. *p<0.05, **p<0.001.

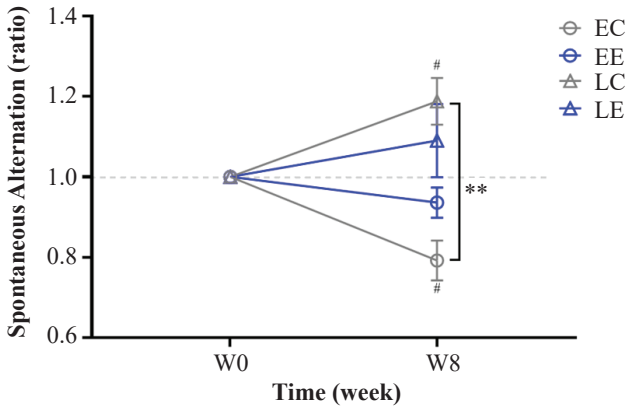

Figure 4. Cognitive performance. Spatial memory performance was assessed using a spatial recognition memory task was assessed at baseline (W0) and week-8 (W8). Y-maze performance was expressed relative to baseline, reflecting the magnitude and direction of change in spontaneous alternation over time rather than absolute spatial working memory capacity. Mixed-design ANOVA was used to analyze (n=4 for LC; n=5 for EC, EE and LE groups). Greenhouse–Geisser corrections were applied when sphericity was violated, and Bonferroni-adjusted post-hoc comparisons were used. Data are presented as mean±SEM. $^{\#}p<0.001$ *vs.* respective W0; $*p<0.05$, $**p<0.001$.

The non-consistent pattern of week-to-week body weight gain observed across groups indicate overall stabilization of body weight prior to load exposure.

Grip strength increased across all groups, indicating neuromuscular adaptation to repeated resistance exercise. In the early-active phase, the exercise group (EE) outperformed controls (EC), suggesting a stronger effect of progressive overload over time, whereas the EC decline at week-4 likely reflects insufficient stimulus. EE showed accelerated early strength gains, improving from week-0 to week-4 and exceeding LE at week-4. Early-active phase exercise may further enhance adaptations by aligning training stimuli with circadian rhythms, consistent with reports of greater endurance gains with early-active exercise.(29) Low continuous loading improved grip strength by week-8, with LC showing greater gains than EC despite identical, non-progressive training. This indicates that circadian timing alone can influence neuromuscular performance, even without progressive overload.(30) Training at ZT1 (LC) appears to enhance adaptation efficiency under low-load conditions, highlighting that optimizing training time may

Table 1. Descriptive statistics for all measured parameters across groups and time points.

Parameter and Time of Observation	EC		EE		LC		LE	
	Mean±SD	CV (%)	Mean±SD	CV (%)	Mean±SD	CV (%)	Mean±SD	CV (%)
Body Weight (g)								
WI	267.44±23.98	8.97	266.56±24.03	9.01	260.78±14.97	5.74	259.22±13.19	5.09
W0	283.78±21.86	7.70	297.33±25.13	8.45	282.33±22.97	8.14	287.33±16.95	5.90
W1	348.00±29.20	8.39	352.00±29.03	8.25	343.22±23.98	6.99	340.89±21.35	6.26
W2	356.33±31.41	8.82	359.89±31.48	8.75	355.22±22.81	6.42	352.00±21.87	6.21
W3	374.44±33.03	8.82	377.33±38.29	10.15	366.22±20.02	5.47	362.67±21.67	5.98
W4	384.00±32.26	8.40	389.33±38.36	9.85	378.44±28.84	7.62	376.11±21.37	5.68
W5	391.11±37.73	9.65	393.78±39.76	10.10	389.44±26.04	6.69	385.33±22.41	5.81
W6	403.44±35.29	8.75	408.00±41.36	10.14	396.44±22.78	5.75	394.89±24.51	6.21
W7	400.33±35.72	8.92	414.89±49.48	11.93	405.89±24.66	6.08	401.33±23.62	5.88
W8	412.00±38.27	9.29	417.67±49.83	11.93	413.78±24.95	6.03	405.78±25.00	6.16
Grip Strength (g)								
W0	45.57±2.11	4.63	44.08±2.03	4.60	36.93±3.21	8.70	36.37±3.25	8.93
W4	37.39±3.83	10.23	57.41±9.41	16.39	49.80±2.46	4.94	48.12±6.68	13.88
W8	136.95±4.97	3.63	152.64±4.66	3.05	143.99±4.00	2.78	142.71±6.62	4.64
Visceral Adiposity (g)								
W8	6.43±1.01	15.64	3.01±0.45	14.79	4.09±0.61	15.00	3.49±0.65	18.53
Hippocampal MDA Level (nmol/g)								
W8	26.30±5.15	19.57	21.88±2.11	9.65	21.73±4.20	19.31	30.22±6.74	22.32
Hippocampal GSH Level (nmol/g)								
W8	1.60±0.14	8.75	19.01±3.96	20.81	1.49±0.20	13.54	6.16±0.35	5.62
Spontaneous Alternation								
W8	0.79±0.11	13.96	0.94±0.08	8.96	1.19±0.12	9.78	1.09±0.20	18.66

improve functional outcomes even when exercise load is minimal.

Evidence on chrono-specific resistance exercise and visceral adiposity remains limited compared with aerobic models. In controls, late-active training (LC) showed lower visceral fat than early-active training (EC) despite identical intensity, suggesting a timing effect independent of load. However, constant-load resistance exercise is more likely to limit fat accumulation than induce lipolysis (31), and similar reductions with late-active exercise have been reported (32). The greater reduction in EE versus EC likely reflects progressive overload rather than timing alone. These findings suggest that load progression appears to be the primary driver of visceral fat reduction, with exercise timing acting as a secondary modulatory factor under constant-load conditions, providing mechanistic insight into muscle–adipose crosstalk and depot-specific adaptation.

Visceral fat was comparable between LC and LE, whereas hippocampal MDA was higher in LE, indicating that oxidative status is shaped by factors beyond adiposity, including exercise load and timing. MDA reflects lipid peroxidation and GSH antioxidant capacity; together, they indicate redox balance. Progressive resistance exercise increased oxidative challenge, reflected by elevated MDA, alongside adaptive antioxidant responses, consistent with load-dependent hormesis.(33) However, the hormetic zone of hippocampal oxidative stress is likely dynamic rather than fixed, with circadian clock mechanisms regulating synaptic plasticity (34) and redox homeostasis (35) suggest that the threshold for beneficial versus detrimental oxidative signalling may vary between early- and late-active phases, thereby influencing the magnitude and direction of adaptive responses to exercise. The lower MDA in LE supports a circadian influence on oxidative plasticity (36), while similar MDA between EE and LC indicates that load and timing can produce convergent redox outcomes. Supporting evidence shows early-phase treadmill exercise enhances brain antioxidant enzymes relative to late-phase training, reinforcing time-of-day effects on hippocampal redox regulation.(37)

After 8 weeks of resistance exercise, spatial working memory exhibited a clear circadian-dependent pattern. Although EC and LC were both exposed to the same sham protocol with constant minimal loading, EC showed a decline in spatial working memory whereas LC improved over time. This pattern suggests that even low-level activity and routine handling may still affect biological processes depending on the chrono-exercise. Specifically, exposure during the early-active phase may have represented a mild disruptive stimulus for hippocampal-dependent performance, while the same exposure during the late-active phase may have been better tolerated or more favorably aligned with the animals internal physiology.(38) Nevertheless, this interpretation should be made cautiously as stress biomarkers were not assessed. Therefore, the observed effects may reflect circadian differences in handling sensitivity, arousal, or other time-dependent neurobehavioral mechanisms. Future studies should include corticosterone, anxiety-related measures, and clock or hippocampal plasticity markers to clarify the underlying processes. Although EE exhibited a more favourable redox profile than LE, this did not translate into improved cognition, suggesting that oxidative adaptations alone are insufficient to predict hippocampal-dependent outcomes. Overall, these findings indicate that circadian timing primarily governs cognitive directionality, followed by modulation of redox status and loading strategy.

This study does not model the altered redox regulation and cognitive deficits seen in metabolic or neurodegenerative conditions. Thus, translation to clinical populations should be cautious. Evidence from aged models further suggests disrupted circadian organization and redox balance, indicating that chrono-dependent adaptations may be attenuated or shifted with aging.(39) Nevertheless, caution is needed when translating these findings to humans, as ZT in rodents represents broad segments of the light–dark cycle rather than precise biological time points, and human circadian rhythms are more flexible and behaviorally influenced than this fixed experimental framework. Although sample size was determined a priori, near-significant outcomes warrant cautious interpretation and require confirmation in larger cohorts. Nonetheless, these findings support chrono-resistance exercise as a modulator of physiological and cognitive outcomes and justify further investigation in aging and disease models, particularly focusing on hippocampal clock gene mechanisms (e.g., brain and muscle Arnt-like protein 1 (BMAL1) and period circadian protein homolog 2 (PER2)) underlying phase-dependent cognitive adaptations.

Conclusion

Early-active phase chrono-resistance exercise (EE) resulting in greatest grip strength adaptations, reduced visceral adiposity, and improved hippocampal redox balance compared with late-active exercise, whereas late-active control (LC) was associated with better preservation and enhancement of spatial working memory. Peripheral

adaptations were primarily driven by progressive loading, with circadian timing acting as a secondary modulatory factor for muscle performance and metabolic outcomes. In contrast, hippocampal-dependent cognitive outcomes were predominantly influenced by circadian timing, with late-active phase exposure improving spatial memory despite weaker redox responses. This result suggests that exercise timing shapes metabolic–brain adaptations.

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Authors Contribution

NAA, NAK, NHSMN, MHFLM, FWI, MHZF, AHAA, DKJ, HAA, NFR and AFML were involved in concepting and planning the research, NAA, NHSMN, MHFLM, MHZF, AHAA and DKJ performed the data acquisition/collection, NAA, NHSMN, MHFLM and AFML calculated the experimental data and performed the analysis, NAA drafted the manuscript and designed the figures, NAA, NAK, NHSMN, MHFLM, FWI, MHZF, AHAA, DKJ, HAA, NFR and AFML aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

Ethical Statement

This study protocol has been approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (Code: FSK/2022/ARIMI FITRI/26-JAN./1228-JAN.-2022-OCT.-2023).

Conflict of Interest

Authors declare no conflict of interest.

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