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Effects of Combined exercise Training With Gamma-Oryzanol Supplementation on Apelin, Asprosin, Insulin Resistance and Visceral Adiposity Indices in Men With Type 2 diabetes: a randomized controlled trial

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Short title:
effect of combined exercise and gamma oryzanol on type 2 diabetes

Effects of Combined exercise training with Gamma-Oryzanol supplementation on apelin, asprosin, Insulin resistance and Visceral Adiposity Indices in men with type 2 diabetes: a randomized controlled trial

Abstract

Introduction: T2DM is a metabolic disorder marked by insulin resistance and adipose tissue dysfunction. This study investigated whether 8 weeks of combined training, with gamma-oryzanol, improves apelin, asprosin, insulin resistance, LAP in men with T2DM

Methods: In this randomized controlled trial study, 30 men with T2DM (aged 45–60 years) were randomly allocated to three groups: combined training+Placebo (CT+Plc, n=10), combined training+gamma-oryzanol (CT+Supp, n=10) and control (CONT, n=10). Training groups performed progressive combined aerobic (50–70% HRmax) and resistance (40–70% 1RM) exercise, 3 sessions/week for 8 weeks. The CT+Supp group additionally consumed 600 mg/day of γ-ORZ. Blood samples were collected at baseline and 48 hours post-intervention following a 12-hour overnight fast.

Results: Both CT+Plc and CT+Supp groups showed significant reductions in apelin, asprosin, FBS, insulin, HOMA-IR compared with baseline ($P<0.05$). Significant differences between Ct+Plc and CT+Supp groups (time × group interaction) were observed for HOMA-IR ($P<0.001$, partial $\eta^2=0.917$), HbA1c ($P<0.001$, partial $\eta^2=0.902$), LAP ($P<0.001$, partial $\eta^2=0.699$) and VAI ($P<0.001$, partial $\eta^2=0.799$). post-hoc comparisons confirmed that CT+Supp produced significantly greater improvements than CT+Plc for these variables (all $P<0.05$). In contrast, no significant differences were found between CT+Plc and CT+Supp for apelin ($P=0.627$), asprosin ($P=0.583$) and insulin ($P=0.092$). Both intervention groups showed significant reductions in apelin, asprosin, HOMA-IR and LAP compared with the control group (all $P<0.05$).

Conclusions: Combined training significantly improved adipokine profiles and metabolic indices in men with T2DM. These findings support combined training as an evidence-based, non-pharmacological strategy for T2DM management. Gamma-oryzanol supplementation demonstrated a potentially additive role particularly in modulating visceral adiposity markers (LAP, VAI), HbA1c, and HOMA-IR warranting investigation in larger, longer-duration trials.

Trial registration: The trial is registered in the Iranian Registry of Clinical Trials (IRCT20251205068219N1) (https://www.irct.ir/trial/87970) on 2026-05-05 in accordance with the Declaration of Helsinki.

Keywords: Combined training; Gamma-oryzanol; Apelin; Asprosin; Lipid accumulation product; Visceral adiposity index; Type 2 diabetes mellitus

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most prevalent and burdensome chronic metabolic disorders worldwide, characterized by progressive hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both (1). The global burden of T2DM is escalating at an alarming rate, with lifestyle factors such as physical inactivity, high-fat dietary patterns, and chronic psychological stress being major contributors to its onset and progression (2).

Adipose tissue particularly visceral adipose tissue (VAT), functions not merely as an energy reservoir but as a highly active endocrine organ. In obesity-associated T2DM, hypertrophied and dysfunctional adipocytes secrete a range of bioactive molecules collectively termed adipokines that profoundly alter insulin signaling, promote chronic low-grade inflammation, and impair beta-cell function (3). Among these, asprosin and apelin have recently attracted considerable research attention for their roles in glucose homeostasis and metabolic regulation.

asprosin is a novel fasting-induced protein hormone encoded by the FBN1 gene and secreted predominantly by white adipose tissue. It exerts orexigenic and glucoregulatory effects via hepatic cAMP–PKA signaling, stimulating gluconeogenesis, and through activation of AgRP neurons in the hypothalamus to increase appetite. Elevated circulating asprosin levels have been consistently reported in individuals with T2DM, obesity, and impaired glucose tolerance, positioning it as a relevant biomarker and potential therapeutic target (4,5). Apelin, the endogenous ligand for the APJ receptor, is an adipokine secreted by adipocytes, cardiomyocytes, and endothelial cells. While apelin initially demonstrates insulin-sensitizing properties by enhancing glucose utilization and oxidative metabolism in skeletal muscle, its sustained elevation in hyperinsulinemic and obese states contributes to downstream insulin resistance through interference with intracellular signaling cascades including PI3K/Akt pathways and beta-cell dysfunction (6,7). This paradoxical behavior makes apelin a compelling target in T2DM research.

Beyond traditional markers such as fasting glucose and HbA1c, composite adiposity indices have emerged as superior metabolic risk assessment tools. The Lipid Accumulation Product (LAP), derived from waist circumference and fasting triglyceride levels, reflects excess intra-abdominal lipid accumulation and has demonstrated strong associations with insulin resistance and T2DM risk (8,9). The Visceral Adiposity Index (VAI), computed from BMI, waist circumference, triglycerides, and HDL-cholesterol, provides an integrated measure of visceral adipose tissue dysfunction and cardiometabolic risk (10). Both LAP and VAI have been shown to outperform BMI alone in discriminating individuals with prediabetes and T2DM and are positively correlated with HOMA-IR (11,12). Physical exercise is a cornerstone of T2DM management. Combined (concurrent) training integrating aerobic and resistance components has shown superior benefits in glycemic control, body composition, and insulin sensitivity compared to either modality alone (13,14). A network meta-analysis demonstrated that combined training produces greater reductions in HbA1c than aerobic or resistance training independently and confers improvements in cardiorespiratory fitness and muscular strength (15). Exercise training has also been shown to significantly reduce both VAI and LAP in patients with T2DM, consistent with improvements in visceral fat and lipid profiles (16).

Gamma-oryzanol (γ-ORZ), a bioactive ester complex derived from rice bran oil, has gained increasing interest as a nutraceutical agent for metabolic syndrome management. Mechanistically, γ-ORZ activates

AMP-activated protein kinase (AMPK), upregulates peroxisome proliferator-activated receptor-alpha (PPARα), inhibits nuclear factor-kappa B (NF-κB), and promotes GLUT4 translocation in skeletal muscle and adipocytes (17). Preclinical and clinical evidence indicates that γ-ORZ reduces total cholesterol (10–15%), LDL-C (8–12%), triglycerides (10–18%), fasting glucose (10–25 mg/dL), and HbA1c (0.3–0.8%) (18). Additionally, γ-ORZ attenuates endoplasmic reticulum stress in pancreatic beta-cells and modulates dopamine D2 receptor signaling to potentiate insulin secretion (19).

Mechanistically, combined exercise training and γ-ORZ supplementation may act through complementary cellular pathways: exercise primarily activates AMPK via an increased skeletal muscle AMP/ATP ratio, whereas γ-ORZ independently potentiates this pathway through its antioxidant and anti-inflammatory properties. Therefore, their combination is theoretically expected to produce an additive metabolic benefit beyond either intervention alone. However, no study to date has simultaneously examined this hypothesis in men with T2DM by assessing apelin, asprosin, HOMA-IR, LAP and VAI. Therefore, the objective of this study was to determine the effects of 8 weeks of combined aerobic-resistance training, with and without gamma-oryzanol supplementation on apelin, asprosin, insulin resistance and visceral adiposity markers in men with T2DM. Accordingly, it was hypothesized that combined training with γ-ORZ supplementation (CT+Supp) would produce greater improvements in apelin, asprosin, insulin resistance and visceral adiposity indices compared with combined training alone (CT+Plc) in men with T2DM.

2. Materials and Methods

2.1 Study Design and Participants

This study was a randomized controlled trial (RCT) with pre/post-testing wich was performed in accordance with the declaration of Helsinki.

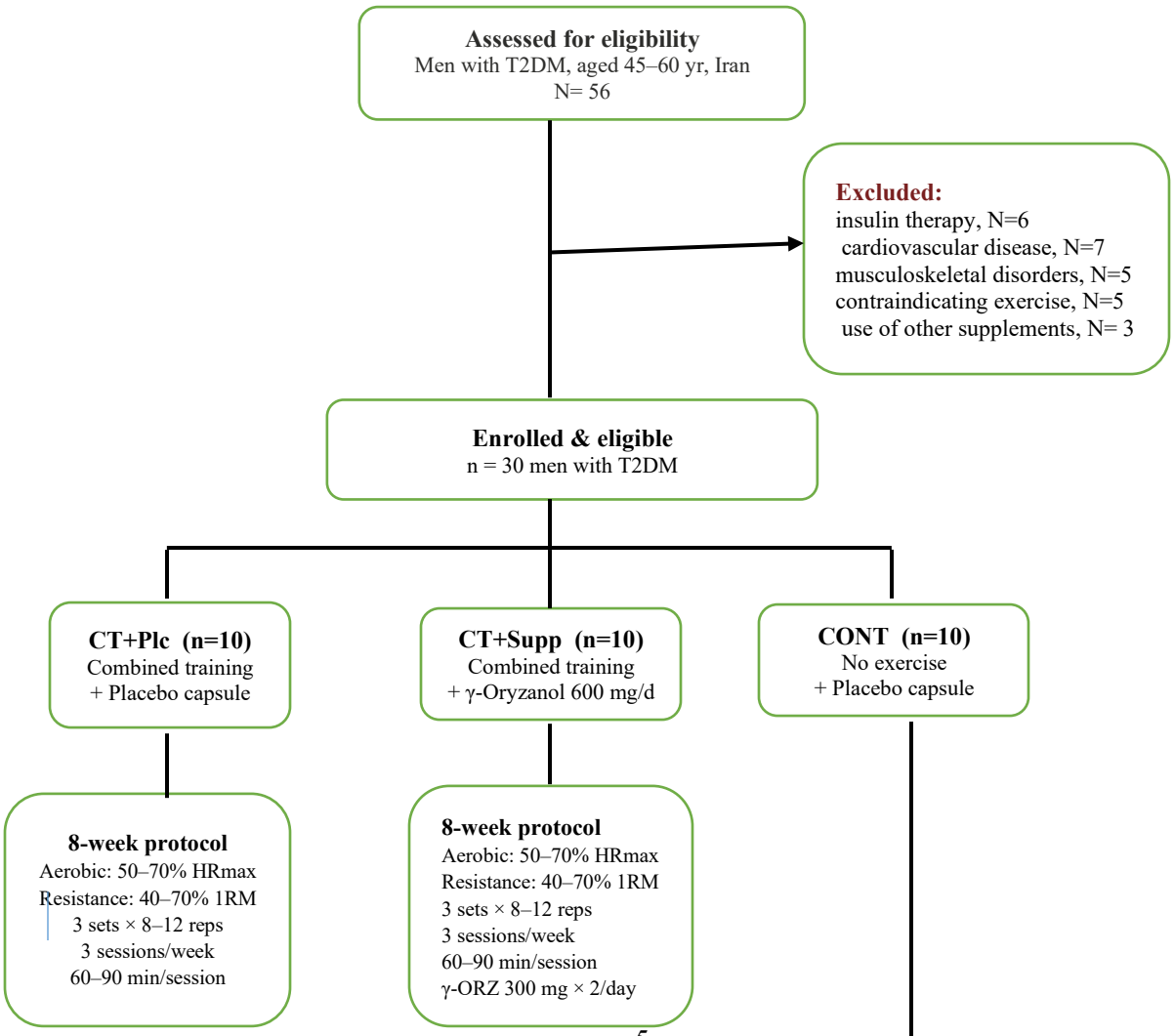
The study population comprised men aged 45–60 years with a confirmed diagnosis of T2DM residing in Iran. Eligibility criteria included: confirmed T2DM diagnosis by a physician, HbA1c ≥ 6.5%, exclusive use of metformin for glycemic management, absence of any structured physical activity program for at least six months and not following any specific dietary regimen at enrollment. Exclusion criteria included insulin therapy, cardiovascular disease, musculoskeletal disorders contraindicating exercise and use of other supplements.

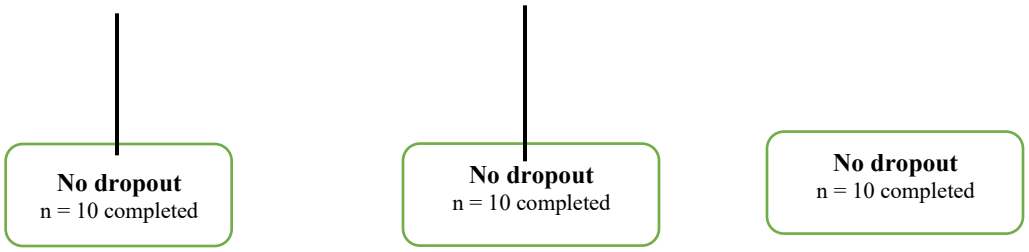
Following initial screening, 30 eligible participants were randomly allocated to three groups: (1) Combined Training + Placebo group (CT+Plc, n=10), (2) Combined Training plus Gamma-Oryzanol Supplementation group (CT+Supp, n=10) and (3) Control group (CONT, n=10). Sample size was calculated using G*Power software (version 3.1.9.7) for a repeated-measures ANOVA with within-between interaction. Assuming a medium effect size ($f = 0.25$, $\alpha = 0.05$, statistical power = 0.80) 3 groups, 2 measurement time points (pre- and post-intervention) the minimum required sample size was determined to be 30 participants (n = 10 per group), and a correlation among repeated measures of $r = 0.5$ the minimum required sample size was 30 participants (n = 10 per group). Participants were randomly allocated using a computer-generated random sequence (simple randomization, 1:1:1) created by an independent researcher not involved in the study. Allocation concealment was ensured using sequentially numbered, opaque, sealed envelopes. Outcome assessors and the statistical analyst were blinded to group allocation. Due to the nature of the exercise intervention, participants and trainers could not be fully blinded; however, placebo capsules were identical in appearance to γ-ORZ tablets. All participants provided written

informed consent prior to enrollment. All 30 enrolled participants completed the 8-week intervention with no dropouts (Figure 1). Exercise adherence was monitored by attendance logs; mean attendance was 95.8% (range 92–100%). Supplement adherence was assessed by returned pill counts and exceeded 96% in both CT+Supp and placebo groups.

All procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki. The study protocol was approved by the ethics committee of Sport Sciences Research Institute (Approval No: IR.SSRC.REC.1404.136). Prior to recruitment, all participants and their legal guardians were thoroughly informed about the study's objectives, experimental procedures, potential risks, and their right to withdraw at any time without penalty. Participation was strictly voluntary; prior to study commencement, written informed consent was obtained from legal guardians and assent from participants. This trial was registered on 05/05/2026 at the Iranian Registry of Clinical Trials (IRCT20251205068219N1)(https://www.irct.ir/trial/87970). This randomized controlled trial was conducted and reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines. No modifications were made to the study protocol or outcome measures between trial commencement and registration.

Fig 1. Consort flowchart of participants





2.2 Exercise Training Protocol

Both training groups performed a progressive combined exercise program comprising 3 sessions per week for 8 weeks. Each session lasted 60–90 minutes, including a 10-minute warm-up, aerobic exercise, a 3–5 minute rest period, resistance exercise, and a 5–10 minute cool-down. Aerobic intensity progressed from 50% to 70% of maximum heart rate (HRmax), and resistance training intensity progressed from 40% to 70% of one-repetition maximum (1RM), with 8–12 repetitions per set across 3 sets. Maximum heart rate (HRmax) was estimated using the age-predicted equation (HRmax = 220 − age). Aerobic exercise intensity was prescribed relative to the estimated HRmax and continuously monitored in real time using Polar chest-strap heart-rate monitors; heart-rate data were recorded and workloads were adjusted as needed to maintain the target intensities. one-repetition maximum (1RM) was estimated using the Brzycki equation. each participant selected a load they could perform for up to 10 repetitions. the attempt repeated until a load producing ≤10 repetitions was identified. The final load and repetition count were recorded and used to calculate 1RM as:

$$1RM\ (kg) = \text{weight}\ (kg)/1.0278 - (0.0278 \times \text{repetitions}).$$

six exercises were included in each session in the following sequence: bench press, leg curl (machine), biceps curl, leg press, barbell shoulder press, leg extension (machine). Sessions were supervised by certified exercise professionals to ensure correct technique and participant safety (20).

The control group maintained their habitual lifestyle without any structured exercise. The progressive training protocol is detailed in Table 1.

Table 1. Progressive Combined Exercise Training Protocol

Exercise Type	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7–8
Aerobic Duration (min)	10	15	20	20	25	25	25–30
Aerobic Intensity (% HRmax)	50%	50%	55%	55%	60%	65%	70%
Resistance Duration (min)	30	30	30	30	35	35	40
Resistance Intensity (% 1RM)	40%	40%	50%	50%	60%	60%	70%
Sets × Repetitions	3×8–12	3×8–12	3×8–12	3×8–12	3×8–12	3×8–12	3×8–12

2.3 Supplementation Protocol

Participants in the CT+Supp group consumed two 300 mg gamma-oryzanol tablets (total 600 mg/day) with breakfast and dinner for 8 weeks (21). The CT+Plc and CONT groups received starch-based placebo capsules that were identical in size, shape, and color to the active supplement to maintain rigorous blinding. Adherence to the supplementation protocol was monitored through bi-weekly pill counts of the returned containers; notably, no serious adverse events were reported throughout the intervention period. All participants were instructed not to alter their habitual dietary patterns during the intervention period.

2.4 Measurement of Outcome Variables

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference (WC) was measured midway between the lowest rib and the iliac crest using a flexible tape measure (cm) with the participant in a standing position. The following composite indices were calculated (16):

$$VAI = [WC / (39.68 + 1.88 \times BMI)] \times (TG_{(mmol/l)} / 1.03) \times (1.31 / HDL_{(mmol/l)})$$

$$LAP = (WC - 65) \times TG$$

$$\text{McAuley Index} = \exp\{2.63 - 0.28 \times \ln(\text{fasting insulin}) - 0.31 \times \ln(\text{fasting triglycerides})\}$$

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR):

$$\text{HOMA-IR} = [\text{Fasting Insulin } (\mu U/mL) \times \text{Fasting Glucose } (mg/dL)] / 405$$

2.5 Blood Sampling and Biochemical Analyses

Venous blood samples (5 mL) were collected from the right antecubital vein at 08:00 h following 12 hours of overnight fasting, both at baseline (24 hours before the start of the protocol) and at post-test (48 hours after the final exercise session). Samples were centrifuged at 5,000 rpm for 10 minutes to separate serum, which was stored at −70°C until analysis.

Serum apelin and asprosin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Sunlong Co., China). Apelin kits (Intra- Assay CV<10%, Inter-Assay: CV<12%, Sensitivity =16 pg/ml) Catalogue Number: SL0277Hu and asprosin (Intra- Assay CV<10%, Inter-Assay: CV<12%, Sensitivity =1 pg/ml) Catalogue Number: SL3098Hu.
Serum insulin was quantified by radioimmunoassay (Liaison kits, UK). Fasting plasma glucose (FPG) was determined by the glucose oxidase method (Pars Azmoon kits, Iran). HbA1c was measured using Shield Access kits (UK). Body composition was evaluated using a multifrequency bioelectrical impedance analyzer (Model IOI370; InBody, South Korea).

2.6 Statistical Analysis

Data are expressed as mean ± standard deviation (SD). The Shapiro–Wilk test was used to assess distributional normality. Levene's test was applied to verify homogeneity of variances. Initially, a one-way ANOVA was executed to ensure the absence of significant baseline disparities among the three experimental cohorts. Furthermore, a 3× 2 Repeated Measures ANOVA examined the temporal dynamics of dependent variables. In alignment

with a conservative statistical interpretation, the Group × Time interaction was prioritized as the primary evidence of efficacy; within-group changes were treated as secondary and interpreted with caution in the absence of a significant interaction. Upon detection of a significant main effect or interaction, the Bonferroni- adjusted post hoc test was utilized for pairwise comparisons. The magnitude of intervention effects was quantified using partial eta squared (ηp2), with benchmarks set at small (0.01), medium (0.06) and large (≥0.14). To ensure robustness beyond dichotomous p-values, primary findings are reported alongside their 95% Confidence Intervals (95% CI). Finally, the interrelationships between the magnitude of change (Delta) in variables, were assessed using Pearson's correlation coefficient. All analyses were performed using SPSS version 27.0 (IBM Corp. USA), with the significance threshold set at $P < 0.05$.

3. RESULTS

3.1 Participant Characteristics

Baseline anthropometric and biochemical characteristics did not differ significantly among the three groups (all $P > 0.05$). Mean age was 49.8 ± 4.15 years in the CT+Plc group, 50.2 ± 2.78 years in the CT+Supp group, and 50.4 ± 5.37 years in the CONT group. The Shapiro–Wilk test confirmed normality of all outcome variables (all $P > 0.05$). Pre- and post-intervention anthropometric values are presented in Table 2**.**

Table 2. Baseline and Post-Intervention Anthropometric Characteristics (N=30)

Variable	CT+Plc Pre	CT+Plc Post	CT+Supp Pre	CT+Supp Post	CONT Pre	CONT Post
Age (yr)	49.8±4.15	—	50.2±2.78	—	50.4±5.37	—
Height (cm)	174.5±5.3	—	175.1±4.8	—	174.9±5.1	—
Weight (kg)	95.39±8.43	91.70±7.88	91.23±7.49	87.86±7.64	87.30±4.57	87.05±4.38
BMI (kg/m²)	30.05±2.14	28.74±2.22	29.35±2.15	28.18±1.82	28.50±2.01	28.08±1.49
Body Fat (%)	30.63±3.91	28.68±3.17	28.68±3.05	25.55±2.70	26.98±2.64	26.46±2.22
WHR	0.986±0.053	0.992±0.056	0.996±0.04	0.989±0.02	0.995±0.039	0.992±0.033

Note*: Data are expressed as mean ±SD for continuous data, or N (%) for categorical data.*

Abbreviation*:.CT+Plc: Combined Training + Placebo; CT+Supp: Combined Training + Gamma-Oryzanol; CONT: Control; BMI: Body Mass Index; WHR: Waist-to-Hip Ratio*

3.2 Adipokines and Metabolic Outcomes

Repeated-measures ANOVA revealed a significant time × group interaction for all primary outcome variables ($P < 0.05$). Pre- and post-intervention metabolic outcomes are presented in Table 3.

Table 3. Pre- and Post-Intervention Values of Metabolic and Adipokine Outcomes (Mean ± SD)

Variable	Group	pretest	Posttest	% change	P- value	RM-ANOVA		
						f	P (time × group)	ηp2
Apelin (ng/mL)	CT+supp	9.22±0.77	6.82±1.71	-26.03	<0.001	6.48	0.005	**0.324**
	CT+plc	8.53±0.67	5.82±1.17	-31.77	<0.001			
	Cont	8.89±0.56	8.29±1.52	-6.75	0.145			
Asprosin (ng/mL)	CT+supp	10.67±1.25	8.41±1.37	-21.18	0.003	7.12	0.003	**0.345**
	CT+plc	9.74±1.19	7.80±1.09	-19.92	<0.001			
	Cont	9.50±0.66	9.22±0.80	-2.95	0.085			
Insulin (µU/mL)	CT+supp	9.08±0.28	7.97±0.24	-12.22	<0.001	80.81	<0.001	**0.857**
	CT+plc	8.98±0.34	8.03±0.29	-10.58	<0.001			
	Cont	8.92±0.32	8.89±0.31	-0.34	0.496			
Glucose (mg/dL)	CT+supp	134.80±4.78	107.60±2.67	-20.18	<0.001	122.7	<0.001	**0.901**
	CT+plc	135.80±5.55	114.50±6.91	-15.68	<0.001			
	Cont	135.10±6.13	134.80±5.65	-0.22	0.627			
HOMA-IR	CT+supp	3.01±0.17	2.11±0.09	-29.90	<0.001	149.8	<0.001	**0.917**
	CT+plc	3.00±0.16	2.29±0.20	-23.67	<0.001			
	Cont	2.97±0.21	2.93±0.18	-1.35	0.250			
HbA1c (%)	CT+supp	7.21±0.35	5.94±0.23	-17.61	<0.001	124.1	<0.001	**0.902**
	CT+plc	7.12±0.39	6.45±0.33	-9.41	<0.001			
	Cont	6.85±0.17	6.78±0.20	-1.02	0.066			
LAP	CT+supp	82.01±13.55	66.76±11.50	-18.60	<0.001	31.37	<0.001	**0.699**
	CT+plc	90.78±16.83	79.49±14.20	-12.44	<0.001			
	Cont	70.53±10.94	69.72±9.65	-1.15	0.285			
VAI	CT+supp	6.62±0.77	5.50±0.60	-16.92	<0.001	53.56	<0.001	**0.799**
	CT+plc	6.73±0.70	5.99±0.60	-11.00	<0.001			
	Cont	6.19±0.59	6.20±0.48	+0.16	0.976			
McAuley Index	CT+supp	6.11±0.08	6.50±0.11	+6.38	<0.001	202.4	<0.001	**0.937**
	CT+plc	6.14±0.126	6.41±0.13	+4.39	<0.001			
	Cont	6.21±0.01	6.22±0.10	-1.61	0.287			

***NOTE*: P- value**: *Significant within-group difference.*

***Abbreviation: VIA**: visceral adiposity index. **LAP**: lipid accumulation product. **HOMA-IR**: homeostasis model assessment-insulin resistance*

Bonferroni post-hoc pairwise comparisons revealed that, for apelin, both the CT+Plc group (MD = 2.10, $p = 0.007$, 95% CI [0.49, 3.71]) and the CT+Supp group (MD = 1.79, $p = 0.025$, 95% CI [0.18, 3.40]) differed significantly from the CONT group, with both intervention groups showing significantly greater reductions in apelin than the control group. No significant difference was observed between the CT+Plc

and CT+Supp groups (MD = 0.31, p = 1.00, 95% CI [-1.30, 1.92]), indicating that gamma-oryzanol supplementation did not conferred an additional benefit over combined training alone for this variable. **(Fig 2A).** Also, results showed that Asprosin, both the CT+Plc group (MD = 1.67, p = 0.019, 95% CI [0.22, 3.11]) and the CT+Supp group (MD = 1.98, p = 0.005, 95% CI [0.54, 3.42]) differed significantly from the CONT group, with both intervention groups showing significantly greater reductions in asprosin than the control group. No significant difference was observed between the CT+Plc and CT+Supp groups (MD = 0.314, p = 1.00, 95% CI [-1.12, 1.75]), indicating that gamma-oryzanol supplementation did not conferred an additional benefit over combined training alone for this variable. **(Fig 2B)**

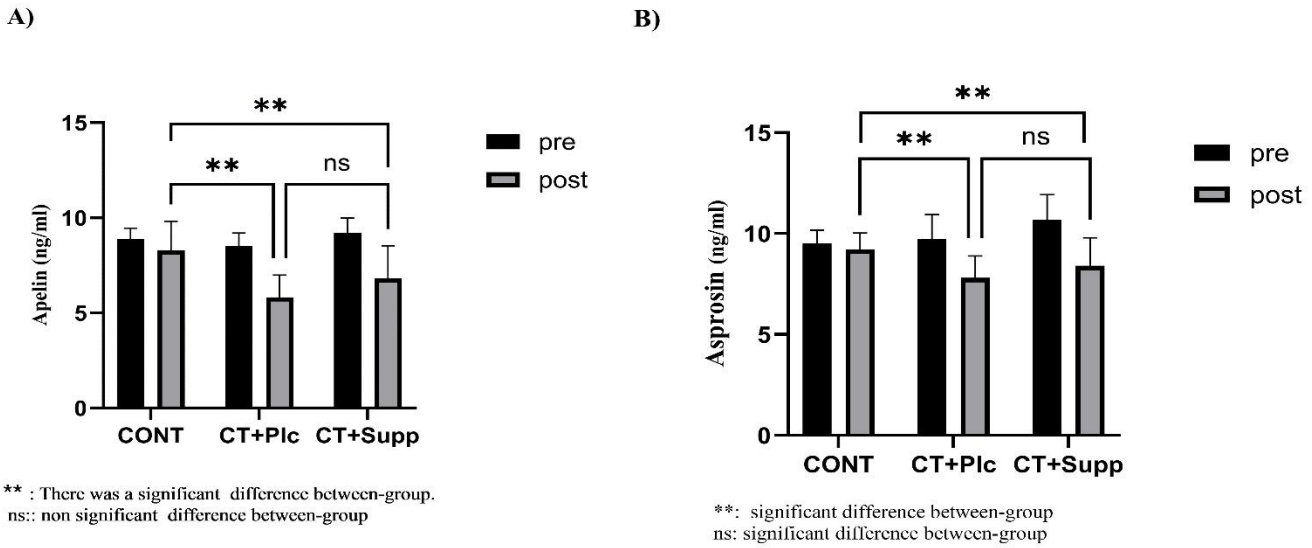


Fig 2A, 2B: Bonferroni post hoc results for apelin and asprosin between groups.

Repeated-measures ANOVA for Apelin: Time effect, $F(1,27) = 54.69$, $P < 0.001$; Group effect, $F(2,27) = 6.061$, $P = 0.007$; Time × Group interaction, $F(2,27) = 6.48$, $P = 0.005$. and for Asprosin: Time effect, $F(1,27) = 41.97$, $P < 0.001$; Group effect, $F(2,27) = 2.018$, $P = 0.153$; Time × Group interaction, $F(2,27) = 7.12$, $P = 0.003$.

Bonferroni post-hoc pairwise comparisons revealed that, for LAP, both the CT+Plc group (MD = 10.46, p = 0.001, 95% CI [5.66, 15.27]) and the CT+Supp group (MD = 14.42, p = 0.001, 95% CI [9.62, 19.23]) differed significantly from the CONT group, with both intervention groups showing significantly greater reductions in LAP than the control group. Also, significant difference was observed between the CT+Plc and CT+Supp groups (MD = 3.96, p = 0.045, 95% CI [0.098, 7.82]), indicating that gamma-oryzanol supplementation conferred an additional benefit over combined training alone for this variable. **(Fig 3A).** Also, results showed that VAI, both the CT+Plc group (MD = 0.873, p = 0.001, 95% CI [0.55, 1.19]) and the CT+Supp group (MD = 1.25, p = 0.001, 95% CI [0.93, 1.57]) differed significantly from the CONT group, with both intervention groups showing significantly greater reductions in VAI than the control group. significant difference was observed between the CT+Plc and CT+Supp groups (MD = 0.38, p = 0.014, 95% CI [0.06, 0.70]), indicating that gamma-oryzanol supplementation conferred an additional benefit over combined training alone for this variable. **(Fig 3B)**

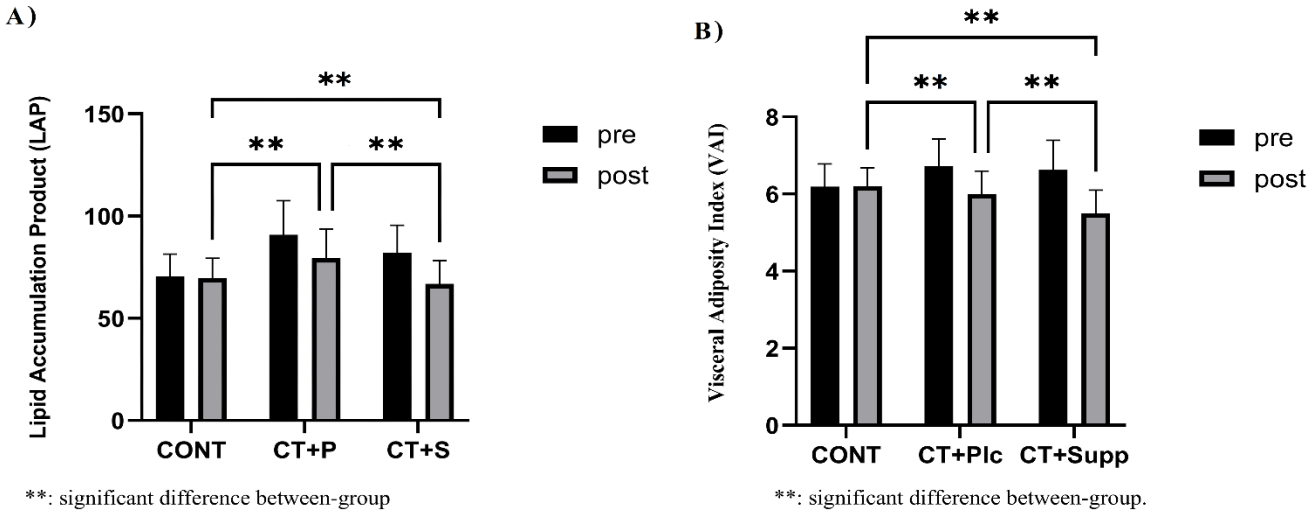


Fig 3A, 3B: Bonferroni post hoc results for LAP and VAI between groups.

Repeated-measures ANOVA for LAP: Time effect, $F(1,27) = 140.70$, $P < 0.001$; Group effect, $F(2,27) = 3.63$, $P = 0.04$; Time × Group interaction, $F(2,27) = 31.37$, $P = 0.001$. and for VAI: Time effect, $F(1,27) = 196.38$, $P < 0.001$; Group effect, $F(2,27) = 0.62$, $P = 0.546$; Time × Group interaction, $F(2,27) = 53.56$, $P = 0.001$.

Significant differences were observed between the CT + plc and CT + Supp groups for HOMA-IR (MD = 0.196, $p = 0.003$, 95% CI [0.062, 0.32]), fasting plasma glucose (MD = 5.90, $p = 0.003$, 95% CI [2.19, 9.60]), McAuley index (MD = 0.129, $p = 0.001$, 95% CI [0.078, 0.17]) and HbA1c (MD = 0.60, $p = 0.001$, 95% CI [0.44, 0.75]) (all $P < 0.05$). indicating that gamma-oryzanol supplementation confer an additional benefit over combined training alone for this variables. In contrast, no significant differences were detected for insulin levels between the two intervention groups (MD = 0.16, $p = 0.092$, 95% CI [0.02, 0.34]). indicating that gamma-oryzanol supplementation did not confer an additional benefit over combined training alone for this variable.

3.3 Correlations Between Variables

Results of Pearson correlate test showed that the Serum apelin levels ($r = 0.460$; $P = 0.011$) and serum asprosin levels ($r = 0.512$; $P = 0.004$) and LAP ($r = 0.790$; $P < 0.001$) were positively associated with HOMA-IR across all participants. Also, results showed that VAI ($r = +0.820$; $P < 0.001$) were positively associated with HOMA-IR. These findings indicate that increase adipose tissue and body weight are associated with greater insulin resistance, independent of group allocation. (Fig 4)

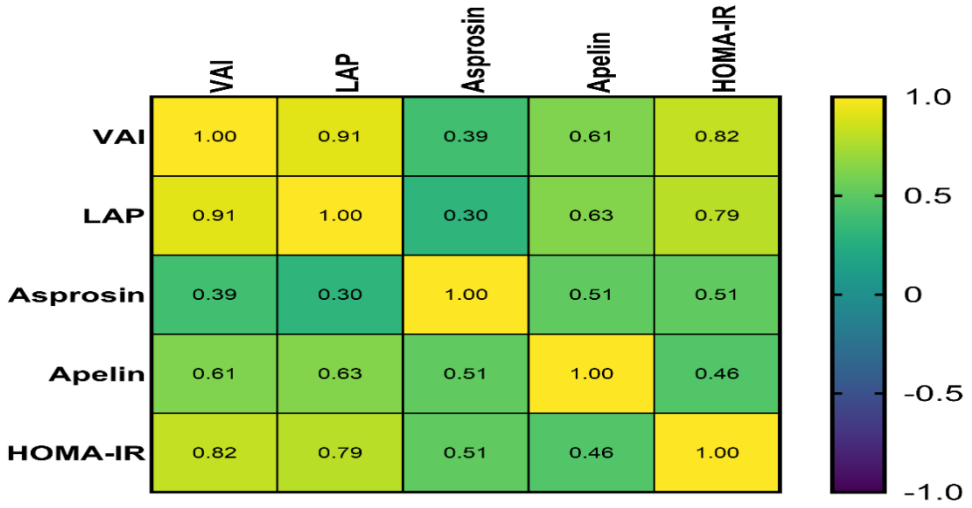


Fig 4. Results of Pearson's correlation analysis examining the relationships among the study variable

4. DISCUSSION

The principal findings of this study demonstrate that 8 weeks of progressive combined training with or without gamma-oryzanol supplementation produced significant improvements in serum apelin, asprosin, fasting plasma glucose, insulin, HOMA-IR, body composition, and visceral adiposity indices (LAP and VAI) in men with T2DM. Although the CT+Supp group consistently demonstrated greater mean reductions across all variables, the between-group difference compared to the CT+Plc group alone did not reach statistical significance for apelin and asprosin, suggesting that combined training exerts the dominant therapeutic effect, with gamma-oryzanol potentially providing a modest additive benefit on select metabolic indices.

The significant reduction in serum apelin observed following eight weeks of combined training is consistent with prior evidence in diabetic populations. Similar reductions in circulating apelin have been reported following aerobic and combined exercise training in both men and women with type 2 diabetes mellitus (22,23). In contrast, Donyaei et al. (2022) observed a significant increase in apelin following combined training and subsequent detraining in women with T2DM (24), whereas Kazemi Nasab et al. (2024), in a systematic review and meta-analysis, found no significant exercise-induced change in this adipokine (25). Such heterogeneity across trials likely reflects the intensity and duration-dependent nature of apelin secretion: low and high intensity training have been shown to elevate circulating apelin without a clear dose–response gradient, suggesting that a secretion threshold, rather than a linear intensity effect, governs its release (26). Consistent with this, aerobic training has been reported to modulate apelin in patients with type 2 diabetes largely independently of parallel changes in glycemic control, indicating that apelin regulation is only partially coupled to insulin sensitivity (27). Collectively, these findings suggest that training modality, session intensity and duration, baseline apelin concentration, and participant adiposity phenotype can each influence the direction and magnitude of the exercise-induced apelin response. In the present study, the observed reduction in apelin paralleled significant decrements in body weight and body fat percentage, a pattern consistent with the proposition that apelin secretion is governed primarily by adipocyte size and hypertrophy rather

than by exercise per se (28). As adipose tissue mass contracts, apelin expression and secretion from adipocytes decline proportionally, attenuating the compensatory hyperapelinemia that characterizes obesity-driven insulin resistance.

Asprosin, a fasting-induced adipokine that enhances hepatic glucose output via the cAMP–PKA–PP2A signaling cascade (4), was significantly reduced in both training groups following the eight-week intervention. These findings align with Ghalavand et al. (2023) and Karimi et al. (2025), who similarly reported exercise-induced reductions in asprosin in men with T2DM (29,30). The underlying mechanism likely involves reductions in visceral adipose tissue mass, the primary source of circulating asprosin (31). With decreased adiposity, asprosin secretion is attenuated, leading to reduced hepatic gluconeogenesis and improved glycemic control (32). Supporting this interpretation, the reduction in asprosin was paralleled by significant decreases in fasting glucose and HOMA-IR across both training groups, reinforcing the proposed link between visceral fat loss, adipokine secretion, and glycemic improvement.

were paralleled by significant decreases in fasting glucose and HOMA-IR across both training groups.

The improvements in insulin resistance observed in this study corroborate a growing body of evidence supporting the efficacy of combined training in T2DM populations. Amaravadi et al. (2024), demonstrated significant improvements in HOMA-IR following 12 weeks of structured exercise in T2DM patients (33), and Valenti et al. (2025) confirmed the benefits of combined aerobic and resistance training in postmenopausal women with T2DM (34). The mechanisms underlying exercise-induced insulin sensitization include upregulation of GLUT4 expression and translocation, enhanced post-receptor insulin signaling (IRS-1/PI3K/Akt pathway), increased skeletal muscle capillarization, and reduced circulating free fatty acids (35). These adaptations collectively reduce hepatic and peripheral insulin resistance, thereby lowering fasting glucose and HOMA-IR.

Regarding visceral adiposity indices, both LAP and VAI decreased significantly in the training groups relative to controls. LAP, derived from waist circumference and fasting triglycerides, directly reflects intra-abdominal lipid accumulation. VAI integrates waist circumference, BMI, triglycerides, and HDL-cholesterol into a sex-specific model that captures adipose tissue dysfunction beyond simple anthropometric measures (10). These indices have demonstrated independent predictive value for T2DM risk, outperforming BMI in several large cohort studies (11,12). Mohammadi et al. (2022), reported significant reductions in both VAI and LAP following 12 weeks of resistance and aerobic training in men with T2DM, consistent with the present findings(16). The exercise-induced reduction in LAP and VAI likely reflects decreased visceral adiposity, improved lipid profiles (particularly triglyceride reduction and HDL elevation), and waist circumference reduction all documented consequences of progressive combined training (36).

The addition of γ-ORZ supplementation (600 mg/day) produced numerically greater reductions across all outcome variables compared to combined training alone, and these differences reached statistical significance for HOMA-IR, HbA1c, LAP, and VAI. This pattern of results is clinically meaningful. Mechanistically, γ-ORZ may potentiate the effects of exercise through complementary anti-inflammatory and antioxidant pathways, including AMPK activation, PPARα upregulation, GLUT4 translocation enhancement, and NF-κB inhibition (17,18). Gamma-oryzanol has also been shown to protect pancreatic beta-cells from endoplasmic reticulum stress and apoptosis (39), potentially enhancing endogenous insulin secretory capacity in T2DM patients. Furthermore, its antihyperlipidemic properties including inhibition

of HMG-CoA reductase and enhanced fecal cholesterol excretion (37). could directly contribute to the observed reductions in LAP and VAI by favorably altering the triglyceride and HDL-C components of these indices. Notably, this pattern contrasts with Nikooyeh et al. (2023), who reported significant improvements in fasting glucose and HbA1c with γ-ORZ-fortified oil in adults with T2DM but no significant change in HOMA-IR (38). This discrepancy may reflect differences in γ-ORZ dose, delivery matrix (capsule vs. dietary oil), and the co-administration of exercise training in the present study, which may act synergistically on insulin signaling pathways. This study has several notable strengths, including its randomized controlled design with concealed allocation, blinded outcome assessment and statistical analysis, 100% adherence with no dropouts, and the simultaneous assessment of multiple adipokine and composite adiposity indices alongside standard glycemic markers an integrative approach rarely applied in previous combined-training trials in T2DM.

Several limitations of this study merit consideration. The relatively small sample size (n = 10 per group) and short intervention duration (8 weeks) represent the principal constraints. These factors may have been insufficient to reveal statistically significant additional benefits of γ-ORZ beyond the dominant exercise effect for adipokine outcomes, potentially contributing to a Type II error. Furthermore, the absence of direct dietary control constitutes a confounding factor that may have differentially affected body composition changes across groups. the fixed γ-ORZ dose (600 mg/day) was based on prior literature rather than a dose-ranging design; given evidence for dose-dependent effects of γ-ORZ on lipid and antioxidant markers, future studies should examine whether higher doses yield greater metabolic benefit when combined with structured exercise. Future studies should employ larger samples, longer intervention periods (≥ 12 weeks), and controlled dietary monitoring. Similar investigations in women and diverse age groups would broaden the generalizability of these findings. Mechanistic investigations using muscle biopsy or adipose tissue gene expression analyses would further elucidate the molecular pathways underlying the observed metabolic adaptations.

5. CONCLUSION

Eight weeks of progressive combined aerobic and resistance training, with or without gamma-oryzanol supplementation, significantly reduced serum Apelin, Asprosin, fasting plasma glucose, serum insulin, HOMA-IR, and visceral adiposity indices (LAP and VAI) in men with T2DM. Although the CT+S group demonstrated consistently greater mean improvements, a statistically significant additional benefit over exercise training alone was observed specifically for HOMA-IR, HbA1c, LAP, and VAI. These findings support combined training as a first-line non-pharmacological intervention for T2DM management and suggest that gamma-oryzanol supplementation may exert a complementary metabolic effect particularly on visceral adiposity and glycemic control warranting further investigation in larger, longer-duration randomized trials.

LIMITATIONS

- The absence of a supplementation only group precluded the independent assessment of gamma oryzanol effects without exercise.
- inability to strictly monitor and control the subjects' nutritional intake.

AUTHOR CONTRIBUTIONS

kazem khodaei and Mehdi rostamizadeh: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; validation; visualization; writing original draft; writing, review and editing.

Hamed hamishehkar: Conceptualization; investigation; methodology; resources; supervision; writing original draft; writing, review and editing.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author, K. Kh, upon reasonable request.

Ethics Declarations

Human ethics and consent to participate

This randomized controlled trial received approval from the Ethics Committee of the Sport Sciences Research Institute (IR.SSRC.REC.1404.136)(https://ethics.ssrc.ac.ir/article_4953.html?lang=en). and was registered with the Iranian Registry of Clinical Trials (IRCT20251205068219N1) (https://www.irct.ir/trial/87970) on 2026-05-05 in accordance with the Declaration of Helsinki. Prior to their participation, all patients were informed about the study's objectives and provided written informed consent.

Protocol and statistical analysis plan

The trial protocol is accessible through the official Iranian Registry of Clinical Trials entry (https://www.irct.ir/trial/87970). No separate published protocol or statistical analysis plan document exists.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the authors used an AI-assisted tool solely for language editing and translation. The authors carefully reviewed, revised, and verified the content after using the tool and take full responsibility for the accuracy and integrity of the final manuscript

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