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Redox-sensitive miRNAs and Humanin could mediate effects of exercise and astaxanthin on oxidative stress and inflammation in type 2 diabetes

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Type 2 diabetes mellitus (T2DM) is driven by oxidative stress (OS) and inflammation (IF), accelerating disease progression. This study examined whether combined aerobic and resistance training (CT) and astaxanthin (AST) supplementation synergistically improve oxidant and inflammatory status as well as metabolic indices in T2DM, focusing on the mediatory role of Humanin (HN) and microRNAs (miRNA-122, miRNA-126-3p, and miRNA-146a). Ninety women with T2DM were randomly assigned to six groups (n = 15 each): control (C), placebo (P), AST supplementation (S), combined training (CT), CT + placebo (CT + P), and CT + AST supplementation (CT + S). CT, CT + P and CT + S groups underwent an 8-week training program (eight exercises, three sessions per week). Groups and CT + S groups received 8 mg/day of AST. OS markers, inflammatory cytokines, HN levels, miRNAs expression, fasting blood glucose (FBG), insulin resistance (HOMA-IR), lipid profile, and hemoglobin A1c (HbA1c) were assessed. Both CT and AST enhanced antioxidant defenses and reduced IF, with CT + S showing the best effects. HN levels increased significantly in CT and CT + S ($p < 0.05$). MiRNA-126-3p and miRNA-146a were upregulated, while miRNA-122 was downregulated in CT + S compared to other groups. Lipid profile improved with both interventions, with CT + S yielding the highest increases in HDL and triglycerides. FBG, IR, and HbA1c improved significantly in CT groups but remained unchanged with S group. The metabolic and anti-inflammatory benefits of CT and AST in T2DM may mediated by the effects of HN on mitochondrial function and insulin signaling, together with miRNA-mediated regulation of lipid metabolism, endothelial health, and innate immunity. Targeting these molecular pathways may improve therapeutic strategies for diabetes management.

Keywords miRNAs, Astaxanthin supplementation, Humanin, T2DM, Combined training

The number of adults living with type 2 diabetes mellitus (T2DM) has increase rapidly over the years. In 2000, around 150 million people were affected, but by 2019, that number had more than tripled to over 450 million. If current trends continue, this figure will rise to about 700 million by 2045. T2DM develops through a combination of factors, including high blood sugar levels, unhealthy fat levels in the blood, oxidative stress (OS), and inflammation (IF)[1]. High blood sugar can lead to the production of harmful free radicals, which can damage lipids, proteins, and even our DNA. Additionally, when pancreatic beta cells are exposed to oxidative stress, it can hinder their ability to produce insulin by affecting the genes responsible for insulin production[2,3]. Furthermore, hyperglycemia-induced OS is thought to elevate the levels of pro-inflammatory proteins and cytokines[4]. Chronic

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IF in insulin-responsive tissues is a key contributor to insulin resistance (IR), and this condition is often linked to elevated levels of reactive oxygen species (ROS)[5]. The interaction of IF and OS results in a worsened vicious cycle that impairs insulin signaling and gives rise to IR[1].

It has been proposed that the most optimal way to manage OS and IF is through a healthy lifestyle[5,6]. Two fundamental parts of a healthy lifestyle include being physically active and having a proper diet. Regular exercise is associated with increased release of anti-inflammatory cytokines and lower concentrations of some pro-inflammatory cytokines[7]. According to Sabouri et al. study[8] the addition of high-intensity interval training (HIIT) to strength training programs significantly improved the patient's IF, antioxidant defense, and glycemic control to the previously existing conditions of the patient. On the other hand, resistance training has gained attention for its benefits in augmenting muscle mass and strength and controlling glucose levels through enhanced insulin sensitivity in the skeletal muscle[9,10] both of which are important in the management of T2DM. Combined aerobic and resistance training (CT) has emerged as combination training due to its multi-faceted benefits which enhance glucose metabolism, IF, and OS[12,13]. Aerobics and resistance training, when done together, provide synergistic advantages which are unique to both forms of training. While CT enhances aerobic fitness, it also enables muscle hypertrophy that improves insulin sensitivity and body composition[14]. Additionally, 8 weeks of combined training also enhanced physical fitness and improved body composition as well as IF[15].

Healthy diet and supplementation are the other frequently used methods in reducing T2DM symptoms[16]. Astaxanthin (AST) is a xanthophyll carotenoid with antioxidant and anti-inflammatory properties, which, unlike those of other carotenoids, can integrate into membrane and scavenge free radicals thanks to its unique molecular structure[17]. Clinical trials showed that AST has the ability to lower oxidative stress, IF, and promote the function of antioxidant enzymes like superoxide dismutase (SOD) and catalase[17]. AST can also improves insulin sensitivity, PI3K/Akt pathways, and also protects the mitochondria, which are relevant in the pathophysiology of T2DM[18,19]. These aspects make AST a promising therapy for the treatment of diabetes and diabetes-related metabolic disorders, especially when combined with lifestyle changes such as structured exercise training.

Previous studies have focused on classical signaling pathways that can explain anti-oxidant and anti-inflammatory effects of exercise and AST. However, there are newly introduced signaling pathways and molecules that can play important role in this process[20,21]. Investigating these novel molecules and their roles in mitigating oxidative stress and inflammation may open promising avenues for both research and therapeutic development. Humanin (HN) is one such recently recognized molecule with a crucial role in reducing OS and IF[22–24]. HN is a member of a class of novel mitochondrial-derived peptides released during mitochondrial dysfunction[25]. HN reduces ROS production, enhances antioxidant protein expression, maintains redox balance[24], and suppresses TNF-α, IL-1β, and IL-6 to inhibit IF[23]. Furthermore, resistance and endurance training has shown to increases HN expression in patients with prediabetes[16]. Exercise—aerobic and endurance- has been shown to increase circulating and skeletal muscle levels of HN, correlating with improved insulin sensitivity and mitochondrial function[26,27]. HIIT is known to have beneficial effects for T2D rats by reducing both IF and OS. Considering what is already known about HN's role in these processes, it seems probable that the benefits of HIIT for T2D rats are due, at least in part, to HN[28].

Another newly discovered molecules with bold role in improving antioxidant and anti-inflammatory response are microRNAs (miRNAs)[29]. miRNA-122, miRNA-126-3p and miRNA-146a showed significant association with inflammatory cytokines as well as IR in diabetes and pre-diabetes patients[30]. MiRNAs also play a crucial role in regulating the molecular mechanisms responsible for β cell dysfunction and their response to OS[31]. MiRNA-146a acts as a negative regulator of inflammation by targeting key pro-inflammatory genes involved in the nuclear factor kappa light chain enhancer of activated B cells (NF-κB) pathway[32]. MiR-146a inhibits overproduction of ROS proinflammatory cytokines[33]. AST supplementation decreased circulating levels of miR-146a and was associated with reduced IL-6 and MDA in T2DM[22]. Two independent reports have shown that aerobic training can increase miR-146a levels[24,25], which may help manage the chronic inflammation often seen in T2DM[25].

MiRNA-122 is specifically expressed in the liver where it is believed to modulate the inflammatory response and insulin resistance in T2D[30]. It is known that increased levels of miRNA-122 are linked to increase IF and OS, which worsens metabolic dysfunction in diabetes[34]. Combined exercise could decrease miRNA-122 expression and, in turn, may improve the components of metabolic syndrome[35]. Downregulation of miR-122 could inhibit OS through PI3K/AKT signaling pathway[34].

Endothelial cells express miRNA-126-3p, and it is important for maintaining vascular health as well as insulin signaling and angiogenesis[36]. Aerobic training[37,38] shown to increase levels of miRNA-126-3p, which improves vascular function and increases insulin sensitivity. Given that insulin signaling and angiogenesis are critical for metabolic balance, the further increase in miRNA-126-3p levels in response to exercise and AST supplementation may help explain the observed therapeutic impact in T2D.

Since both exercise and AST can potentially impact HN and certain microRNAs that regulate OS and IF, our study aimed to explore how the combination of CT and AST affects these processes. Specifically, we looked at how they influence HN, miRNA-122, miRNA-126-3p, and miRNA-146a, with the ultimate goal of reducing the symptoms and complications associated with diabetes.

Material and methods

Experimental procedure

This study was a double-blind, randomized, placebo-controlled trial and 90 volunteer's women with T2DM were enrolled in it. Kurdistan University of Medical Sciences referred the participants and they were selected based on specific inclusion criteria: age between 30 and 60 years old, had a confirmed diagnosis of T2DM with no insulin usage, were not pregnant or breastfeeding, and did not have chronic diseases, cancers, kidney

failure, heart disease, thyroid disorders, or other inflammatory conditions. Additionally, the participants had not used vitamins or antioxidant supplements in the previous 6 months and were non-smokers and non-alcohol consumers. Informed written consent was obtained from each participant before commencing the study procedures. Ethics Committee of Kerman University of Medical Sciences approved all procedure (Approval Code: IR.KMU.REC.1401.530). This study was registered in the Iranian Registry of Clinical Trials (IRCT20230225057524N1) on 20/03/2023. The research was conducted in full compliance with the Declaration of Helsinki, Iranian national research ethics guidelines, and the International Committee of Medical Journal Editors (ICMJE) recommendations for clinical trials.

Participants were randomly assigned to one of the six groups (n = 15 in each): Control (C), placebo (P), AST supplementation (S), Combined training (CT), CT + placebo (CT + P), and CT + AST supplementation (CT + S). The exercise sessions were conducted for 8 weeks, with 3 sessions per week. Before starting the exercise programs, all participants were familiarized with the environment and exercises. Before the study began and 72 h after the final training or supplementation session, we measured the participants' body composition, height, and weight. Each participant also went through initial evaluations by a physical medicine specialist and an endocrinologist. We excluded individuals from the study if they had any of the following conditions: a history of ketoacidosis within the past six months, high blood sugar levels (HbA1c > 10%), diabetic complications affecting small blood vessels, diabetic neuropathy, liver issues (indicated by elevated plasma aminotransferase or gamma-glutamyl transferase levels for their age and gender), kidney problems (with serum creatinine levels above normal for their age and gender), severe anemia, uncontrolled high blood pressure, cardiovascular diseases (such as congestive heart failure or a history of heart attacks), or any cerebrovascular incidents.

Exercise protocol

CT, CT + P, and CT + S groups performed the following exercises three time a week for 8 weeks:

1. Jumping jacks
2. Wall sit/single leg dead lift
3. Push-up
4. Plank
5. Mountain climber
6. Squat
7. Triceps dip
8. Superman

Exercise 1 to 8 were performed sequentially with a 15-s rest between exercises. Completing all exercises (1 to 8) was considered one circuit, and two circuits were completed in each session, with a 3-min rest between circuits. To increase the load, the duration of each exercise was increased as follows[39,40]:

- Weeks 1 and 2: 10 s per exercise
- Weeks 3 and 4: 12 s per exercise
- Weeks 5 and 6: 14 s per exercise
- Weeks 7 and 8: 16 s per exercise

Before starting the exercise program, a 10-min warm-up, including light running and stretching exercises, was conducted, followed by a similar cool-down activity. The intensity of the exercise throughout the session was set at 65% of heart rate reserve (HRR), monitored using a Polar heart rate monitor (polar H10, USA). HRR was calculated by subtracting resting heart rate (RHR) from maximum heart rate (MHR). MHR considered as 207–0.7*age and RHR as the number of heart beats per minute while at complete rest, typically measured first thing in the morning[41].

Supplementation

Active treatment involved the oral administration of 8 mg of AST (Nature Vision; USA), sourced from H. pluvialis (microalgae). The inactive ingredients used for both the supplement and placebo included dicalcium phosphate, microcrystalline cellulose, stearic acid, silicon dioxide, and magnesium stearate. Participants in P, S, CT + P and CT + S were instructed to take one 8-mg tablet of either the placebo (which contained all the ingredients of the supplement except for AST) or the AST supplement immediately after lunch to maximize absorption, for a duration of 8 weeks.

Dietary assessment

3-day food records were used before, during, and after the study period. It included details on the volume or weight and type of food consumed. Nutritionist IV software (Version 4.1, First Databank Division, The Hearst Corporation, and San Bruno, CA, USA) was used to evaluate energy and macronutrient intake. At each bi-weekly visit, participants' adherence to the study protocol was monitored by counting the remaining AST tablets and reminding them to maintain their usual diet, lifestyle, and medication regimen.

RNA extraction and quantitative real-time PCR

Total RNA was extracted using the GeneAll Kit (General Biosystems, Seoul, Korea, Cat# 315–150) in accordance with the manufacturer's guidelines. Thermo Fisher Scientific Nanodrop was used to assess the RNA quality, measuring absorbance at wavelengths of 260 and 280 nm to determine RNA concentration in each sample. The A260/A280 ratio provides a general indication of nucleic acid (RNA) purity. Since the absorbance ratios for the

samples were between 1.8 and 2.2, the RNA purity was confirmed. cDNA synthesis was then performed using Stem-loop quantitative reverse transcription PCR (RT-qPCR) (Bonyakhteh, Tehran, Iran, Cat# BON209001). Following this, the BONmiR QPCR Kit (Bonyakhteh, Tehran, Iran, Cat# BON209002) was employed to analyze miRNA gene expression using the Rotor Gene 6000 machine (Corbett, Concorde, NSW, Australia). The relative levels of miRNAs (fold changes) were calculated using the 2-ΔΔCt method (Livak). The changes in threshold cycle (ΔCt) were determined by subtracting the Ct values of the reference SNORD from the Ct values of the target miRNAs (miRNA-122, miRNA-126-5p, and miRNA-146a). ΔΔCt was then calculated by subtracting the average ΔCt values of the control group (healthy individuals) from those of the case group (pre-diabetes or diabetes). The formula used was ΔΔCt = (Ct miRNA − Ct SNORD) T2DM or pre-diabetes − (Ct miRNA − Ct SNORD) healthy individuals.

To assess the expression levels of miRNA-122, miRNA-126-5p, and miRNA-146a, qPCR was performed on whole blood samples, with SNORD serving as the internal control gene. Amplification efficiency, a critical factor for the accuracy and reliability of real-time qPCR, was calculated from the slope of the log-linear region, similar to that used for standard curves. The primer sets used in this study exhibited efficiencies between 90 and 110%.

Bioinformatics analysis

Bioinformatics tools, including miRDB (http://www.mirdb.org), miRanda (http://www.microrna.org/microrna/), and TargetScan (http://www.targetscan.org), were utilized to identify the potential mRNA targets of miRNA-122, miRNA-126-3p, and miRNA-146a (Table 1).

Metabolic indices measurement

The levels of fasting blood glucose (FBG), insulin, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using the Selectra Autoanalyzer (Pars Azmoon, Iran) with standard commercial kits. The following clinical definitions and reference ranges were considered: FBG measuring between 100 and 125 mg/dL indicates prediabetes, while 126 mg/dL or higher suggests diabetes mellitus. Regarding HbA1c, it indicates the plasma glucose level for the preceding 2–3 months, with pre-diabetes set for 5.7–6.4%. An increase of 6.5% and above indicates diabetes. Insulin Resistance: The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated using the formula:

$$\text{HOMA - IR} = \frac{\text{Fasting Insulin}\ (\mu U/mL) \times \text{Fasting Gulcose}\ (mmol/L)}{22.5}$$

Insulin resistance is suggested with A HOMA-IR > 2.5. Lipid profile, cholesterol: average is < 200 mg/dL, LDL-C: optimal < 100 mg/dL, HDL-C: protective > 50 mg/dL for women, normal < 150 mg/dL for TG[42,43].

These metabolic indicators were selected due to their relevance in evaluating the cardiometabolic and glycemic status of individuals with T2DM. HbA1c was measured using an Adichem device through the Ion Exchange Chromatography method and reported as a percentage.

Inflammation measurement

To assess inflammation, we used ELISA to measure the serum levels of IL-36α, IL-36γ, IL-36Ra, and IL-17. The kits for these measurements were sourced from Cusabio Biotech Co., Ltd., and we conducted the experiments following the manufacturer's guidelines. Each sample was tested twice and in a random order to ensure accuracy.

Statistical analysis

We used descriptive statistics to summarize the participants' characteristics and measurements. We checked the normality of the data using the Shapiro–Wilk test. To analyze differences within groups, we employed ANCOVA (using the pre-test as a covariate) followed by Bonferroni's correction for post-hoc testing. All statistical analyses were conducted using SPSS V22 (SPSS Inc., Chicago, IL), with a significance level set at $p < 0.05$.

Results

Clinical and anthropometric characteristics of the study population

Table 2 details the participant's baseline physical and medical characteristics. As indicated, no statistically significant difference was observed in basic characteristics. In addition, none of the patients took any special medicine for weight loss during the study.

Expression levels of miRNA-122, miRNA-126-3p, and miRNA-146a

The actual values (Mean ± SD) for all measured variables are presented in supplementary table S1. The results indicated a significant difference between groups in miRNA-122 ($F_{(5,83)} = 67.58$, $p < 0.001$, $\eta^2 = 0.756$), miRNA-126-3p ($F_{(5,83)} = 21.69$, $p < 0.001$, $\eta^2 = 0.623$), and miRNA-146a ($F_{(5,83)} = 27.35$, $p < 0.001$, $\eta^2 = 0.677$) expression levels, while adjusting for pre-test. The *post-hoc* test for miRNA-122 showed significantly lower levels in CT + P (d: 4.17, $p < 0.001$), CT (d: 3.52, $p < 0.001$), S (d: 2.74, $p < 0.001$), and CT + S (d: 5.77, $p < 0.001$) compared with CG. *Post-hoc* tests revealed significantly lower levels in CT + P (d: 4.44, $p < 0.001$), CT (d: 3.79, $p < 0.001$), S (d: 3.01, $p < 0.001$), and CT + S (d: 6.04, $p < 0.001$) compared with P. Also, significantly higher levels were observed in S (d: − 2.73, $p = 0.013$), and significantly lower in CT + S (d: 0.27, $p = 0.004$) compared with CT + P. Additionally, the CT + S group showed significantly lower levels (d: 2.25, $p < 0.001$) compared with CT group. A significant lower was observed in CT + S (d: 3.03, $p < 0.001$) compared with S group. No difference was found between the other groups ($p > 0.05$) (Fig. 1).

The post hoc correction test for miRNA-126 revealed significantly higher levels in CT + P (d: − 2.11, $p < 0.001$), CT (d: − 2.36, $p < 0.001$), S (d: − 1.31, $p = 0.030$), and CT + S (d: − 3.29, $p < 0.001$) compared with C

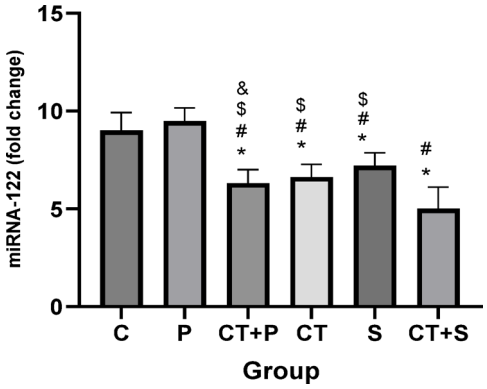

Fig. 1. Comparison of the expression levels (Mean ± SD) of miRNA-122 in post-tests. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. & indicates a significant difference with the S. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S* combined training + supplement.

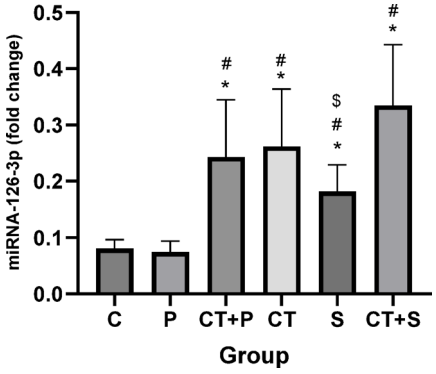

Fig. 2. Comparison of the expression levels (Mean ± SD) of miRNA-126-3p in post-tests. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S* combined training + supplement.

group. *Post-hoc* test revealed significantly higher levels in CT + P (d: − 2.21, $p < 0.001$), CT (d: − 2.46, $p < 0.001$), S (d: − 1.42, $p = 0.015$), and CT + S (d: − 3.39, $p < 0.001$) compared with P group. Additionally, miRNA-126 levels were significantly higher in CT + S (d: − 1.97, $p < 0.001$) compared with S group. No significant differences were observed between the other groups ($p > 0.05$) (Fig. 2).

Bonferroni's *post-hoc* correction test for miRNA-146a revealed significantly higher levels in CT + P (d: − 2.68, $p < 0.001$), CT (d: − 2.72, $p < 0.001$), S (d: − 2.04, $p < 0.001$), and CT + S (d: − 3.67, $p < 0.001$) compared with C group. *Post-hoc* test showed significantly higher levels in CT + P (d: − 2.68, $p < 0.001$), CT (d: − 2.72, $p < 0.001$), S (d: − 2.04, $p < 0.001$), and CT + S (d: − 3.67, $p < 0.001$) compared with P group. Additionally, miRNA-146a levels were significantly lower in the CT + S (d: − 1.63, $p = 0.003$) compared with S group. No significant differences were observed between the other groups ($p > 0.05$) (Fig. 3).

IL-36α, IL-36γ, IL-36Ra, and IL-17 levels

The results of the univariate ANCOVA test showed a significant difference between groups in IL-36α ($F_{(5,83)} = 24.28$, $p < 0.001$, $\eta^2 = 0.453$), IL-36γ ($F_{(5,83)} = 24.38$, $p < 0.001$, $\eta^2 = 0.537$), IL-36Ra ($F_{(5,83)} = 27.88$, $p < 0.001$, $\eta^2 = 0.471$), and IL-17 ($F_{(5,83)} = 17.87$, $p < 0.001$, $\eta^2 = 0.434$) levels, while adjusting for pre-training. Bonferroni's *post-hoc* correction test for IL-36α revealed significantly lower levels in CT + P (d: 1.95, $p < 0.001$), CT (d: 2.42, $p < 0.001$), S (d: 1.95, $p < 0.001$), and CT + S (d: 3.29, $p < 0.001$) compared with C group. *Post-hoc* test revealed significantly lower levels in CT + P (d: 2.30, $p < 0.001$), CT (d: 2.77, $p < 0.001$), S (d: 2.31, $p < 0.001$), and CT + S (d: 3.64, $p < 0.001$) compared with P. Additionally, IL-36α were significantly lower levels in CT + S compared with CT + P (d:1.34, $p = 0.031$), CT (d:1.24, $p = 0.043$), and S (d:1.33, $p = 0.025$). No significant differences were observed between the other groups ($p > 0.05$) (Fig. 4).

Bonferroni's *post-hoc* correction test for IL-36γ revealed significantly lower levels in CT + P (d: 2.43, $p < 0.001$), CT (d: 2.42, $p < 0.001$), S (d: 1.44, $p = 0.012$), and CT + S (d: 3.36, $p < 0.001$) compared with C group. The *Post-hoc* test revealed significantly lower levels in CT + P (d: 2.59, $p < 0.001$), CT (d: 2.58, $p < 0.001$), S (d: 1.60, $p = 0.004$),

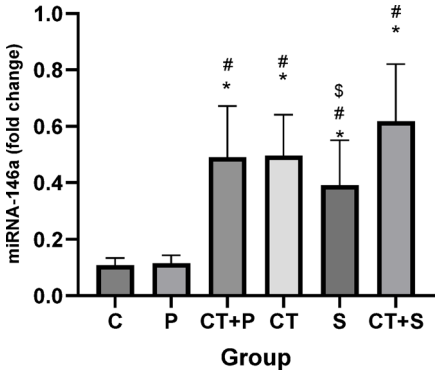

Fig. 3. Comparison of the expression levels (Mean ± SD) of miRNA-146a in post-tests. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. *C* control group, *P* placebo, *CT + P* combined training + placebo, *CT* combined training, *S* supplement, and *CT + S* combined training + supplement.

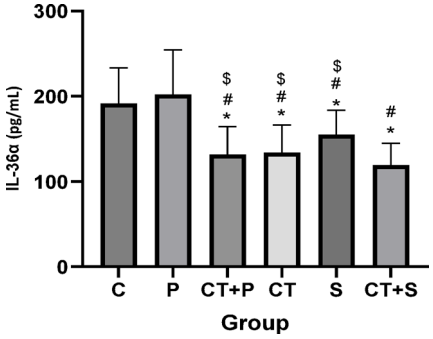

Fig. 4. Serum IL-36α level (Mean ± SD) in post-test. * indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. *C* control group, *P* placebo, *CT + P* combined training + placebo, *CT* combined training, *S* supplement, and *CT + S* combined training + supplement.

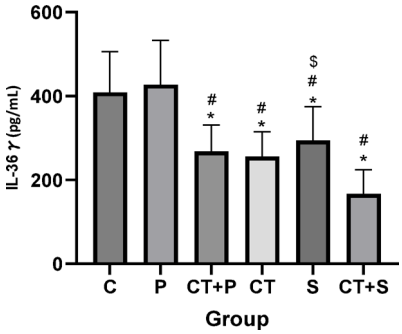

Fig. 5. Serum IL-36γ level (Mean ± SD) in post-test. * indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. *C* control group, *P* placebo, *CT + P* combined training + placebo, *CT* combined training, *S* supplement, and *CT + S* combined training + supplement.

and CT + S (d: 3.52, $p < 0.001$) compared with P group. Also, the *post-hoc* test showed significantly lower levels in CT + S (d: 1.33, $p = 0.025$) compared with S. No difference was found between the other groups ($p > 0.05$) (Fig. 5).

Bonferroni's *post-hoc* correction test for IL-36Ra revealed significantly higher levels in CT + P (d: − 2.52, $p < 0.001$), CT (d: − 2.46, $p < 0.001$), S (d: − 2.25, $p < 0.001$), and CT + S (d: − 3.68, $p < 0.001$) compared with C group. The *Post-hoc* test showed significantly higher levels in CT + P (d: − 2.72, $p < 0.001$), CT (d: − 2.35, $p < 0.001$), S (d: − 2.44, $p < 0.001$), and CT + S (d: − 3.88, $p < 0.001$) compared with P group. Also, IL-36Ra were significantly

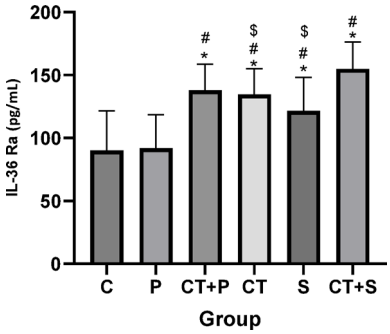

Fig. 6. Serum IL-36Ra level (Mean ± SD) in post-test. * indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. *C* control group, *P* placebo, *CT + P* combined training + placebo, *CT* combined training, *S* supplement, and *CT + S* combined training + supplement.

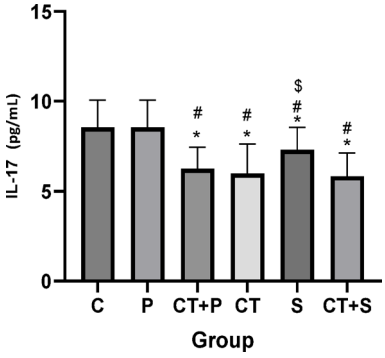

Fig. 7. Serum IL-17 level (Mean ± SD) in post-test. * indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. *C* control group, *P* placebo, *CT + P* combined training + placebo, *CT* combined training, *S* supplement, and *CT + S* combined training + supplement.

higher levels in CT + S (d: − 2.83, $p = 0.006$) compared with CT group. The *post-hoc* test revealed significantly higher levels difference in CT + S (d: − 1.43, $p = 0.014$) compared with S. No difference was found between the other groups ($p > 0.05$) (Fig. 6).

Bonferroni's *post-hoc* correction test for IL-17 revealed significantly lower levels in CT + P (d: 2.18, $p < 0.001$), CT (d: 2.46, $p < 0.001$), S (d: 1.39, $p = 0.017$), and CT + S (d: 2.90, $p < 0.001$) compared with C group. *Post-hoc* test showed significantly lower in CT + P (d: 2.03, $p < 0.001$), CT (d: 2.32, $p < 0.001$), S (d: 1.24, $p = 0.050$), and CT + S (d: 2.75, $p < 0.001$) compared with P group. Also, Bonferroni's correction *post-hoc* test revealed significantly lower levels in CT + S (d: 1.51, $p = 0.007$) compared to S group. No difference was found between the other groups ($p > 0.05$) (Fig. 7).

Total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity

The univariate ANCOVA test showed a significant difference between groups in TAC ($F_{(5,83)} = 44.51$, $p < 0.001$, $\eta^2 = 0.729$), SOD ($F_{(5,83)} = 9.87$, $p < 0.001$, $\eta^2 = 0.338$), and GPx ($F_{(5,83)} = 38.06$, $p < 0.001$, $\eta^2 = 0.629$) levels, while adjusting for the pre-training. Bonferroni's *post-hoc* test for GPx revealed significantly higher levels in CT + P (d: − 3.11, $p < 0.001$), CT (d: − 3.27, $p < 0.001$), S (d:—1.73, $p = 0.001$), and CT + S (d: − 4.13, $p < 0.001$) compared with C group. Post hoc test showed significantly higher levels in CT + P (d: − 3.27, $p < 0.001$), CT (d: − 3.48, $p < 0.001$), S (d: − 1.94, $p < 0.001$), and CT + S (d: − 4.34, $p < 0.001$) compared with P group. Also, GPx were significantly lower levels in S (d: 1.37, $p = 0.019$) compared to CT + P and CT (d: 1.54, $p = 0.006$) groups. The Post hoc test showed significantly higher levels in CT + S compared with S (d: − 2.40, $p < 0.001$). No difference was found between the other groups ($p > 0.05$) (Fig. 8).

Bonferroni's *post-hoc* test for SOD revealed significantly higher levels in CT + P (d: − 1.30, $p = 0.033$), CT (d: − 1.54, $p = 0.006$), and CT + S (d: − 2.23, $p < 0.001$) compared with C group. The *Post-hoc* test showed significantly higher levels in CT + P (d: − 1.43, $p = 0.013$), CT (d: − 1.68, $p = 0.002$), and CT + S (d: − 2.36, $p < 0.001$) compared with P group. No difference was found between the other groups ($p > 0.05$) (Fig. 9).

Bonferroni's *post-hoc* correction test for TAC revealed significantly higher levels in CT + P (d: − 3.34, $p < 0.001$), CT (d: − 3.25, $p < 0.001$), S (d: − 2.20, $p = 0.001$), and CT + S (d: − 4.78, $p < 0.001$) compared with

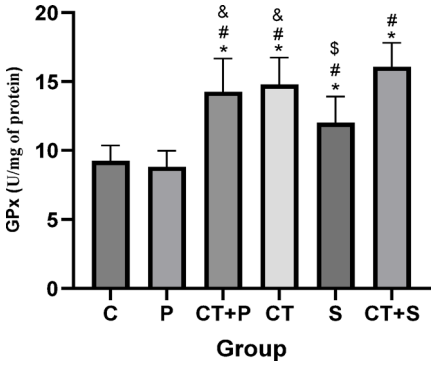

Fig. 8. GPx level (Mean ± SD) in post-test. * indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. & indicates a significant difference with the S. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S* combined training + supplement.

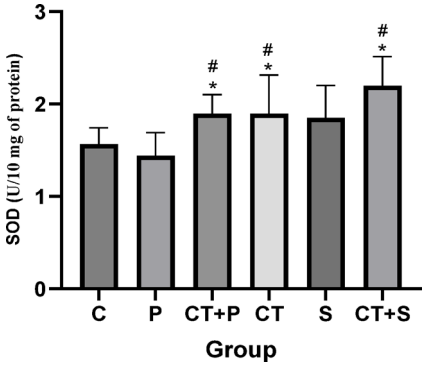

Fig. 9. SOD level (Mean ± SD) in post-test. * indicates a significant difference with the C. # indicates a significant difference with the P. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S* combined training + supplement.

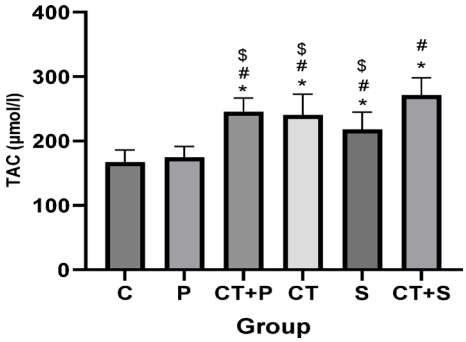

Fig. 10. TAC level (Mean ± SD) in post-test. * indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S* combined training + supplement.

C group. The *Post-hoc* test revealed significantly higher levels in CT + P (d: − 3.30, $p < 0.001$), CT (d: − 3.20, $p < 0.001$), S (d: − 2.16, $p < 0.001$), and CT + S (d: − 4.74, $p < 0.001$) compared with P group. Also, TAC were There were significantly higher in CT + S compared with S (d: − 2.57, $p < 0.001$), CT (d: − 1.53, $p = 0.006$), CT + P (d: − 1.43, $p = 0.013$) groups. No difference was found between the other groups ($p > 0.05$) (Fig. 10).

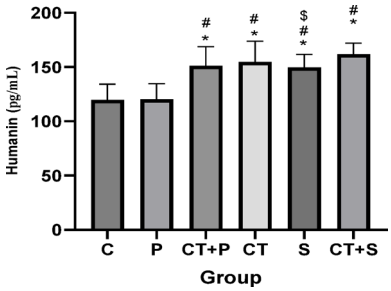

Fig. 11. Serum humanin level ((Mean ± SD). * Indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. *C* control group, *P* placebo, *CT + P* combined training + placebo, *CT* combined training, *S* supplement, and *CT + S* combined training + supplement.

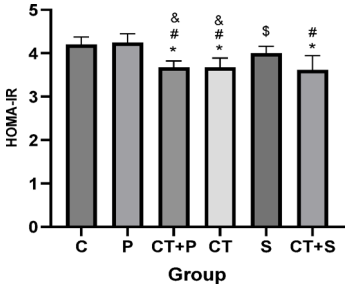

Fig. 12. Serum insulin resistance level (Mean ± SD). * Indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. & indicates a significant difference with the S. *C* control group, *P* placebo, *CT + P* combined training + placebo, *CT* combined training, *S* supplement, and *CT + S* combined training + supplement.

Serum lipid profiles, fasting blood glucose, insulin resistance, HbA1c, and humanin levels

The univariate ANCOVA test showed significant differences between groups in cholesterol ($F_{(5,83)} = 12.21$, $p < 0.001$, $\eta^2 = 0.287$), triglycerides (TG) ($F_{(5,83)} = 23.29$, $p < 0.001$, $\eta^2 = 0.591$), HDL ($F_{(5,83)} = 22.32$, $p < 0.001$, $\eta^2 = 0.382$), fasting insulin ($F_{(5,83)} = 44.51$, $p < 0.001$, $\eta^2 = 0.729$), HbA1c ($F_{(5,83)} = 30.21$, $p < 0.001$, $\eta^2 = 0.677$), fasting blood glucose ($F_{(5,83)} = 45.39$, $p < 0.001$, $\eta^2 = 0.777$), insulin resistance ($F_{(5,83)} = 15.32$, $p < 0.001$, $\eta^2 = 0.281$), and humanin ($F_{(5,83)} = 24.29$, $p < 0.001$, $\eta^2 = 0.460$) levels, while adjusting for the pre-training.

Bonferroni's *post-hoc* correction test for humanin revealed significantly higher in CT + P (d: − 2.69, $p < 0.001$), CT (d: − 2.77, $p < 0.001$), S (d: − 1.86, $p = 0.001$), and CT + S (d: − 3.18, $p < 0.001$) compared with C group. The *Post-hoc* test showed significantly higher in CT + P (d: − 2.74, $p < 0.001$), CT (d:—2.88, $p < 0.001$), S (d: − 1.91, $p < 0.001$), and CT + S (d: − 3.23, $p < 0.001$) compared with P group. Also, humanin were significantly higher in CT + S compared with S group (d: − 1.31, $p = 0.030$). No difference was found between the other groups ($p > 0.05$) (Fig. 11).

Bonferroni's *post-hoc* test for HOMA-IR showed significantly lower in CT + P (d: 3.29, $p < 0.001$), CT (d: 3.32, $p < 0.001$), and CT + S (d: 3.46, $p < 0.001$) compared to C group. *Post-hoc* test revealed significantly lower in CT + P (d: 3.52, $p < 0.001$), CT (d: 3.55, $p < 0.001$), and CT + S (d: 3.70, $p < 0.001$) compared with P group. The *post-hoc* test showed significantly lower in S compared to CT + P (d:—2.39, $p < 0.001$), and CT (d:—2.42, $p < 0.001$) groups. Also, HOMA-IR were significantly lower in CT + S compared with S (d: 2.56, $p = 0.030$) group. No difference was found between the other groups ($p > 0.05$) (Fig. 12).

Bonferroni's *post-hoc* test for HbA1c showed significantly lower in CT + P (d: 2.43, $p < 0.001$), CT (d: 2.20, $p < 0.001$), and CT + S (d: 4.24, $p < 0.001$) compared to C group. The *Post-hoc* test showed significantly lower in CT + P (d: 2.12, $p < 0.001$), CT (d: 1.89, $p < 0.001$), and CT + S (d: 3.93, $p < 0.001$) compared with P group. The *post-hoc* test showed significantly higher in S compared with CT + P (d: − 1.43, $p = 0.014$), and CT (d: − 1.19, $p = 0.049$) groups. Also, HbA1c were significantly lower in CT + S compared with S (d: 3.24, $p < 0.001$), and CT (d: 2.04, $p < 0.001$) groups. No difference was found between the other groups ($p > 0.05$) (Fig. 13).

Bonferroni's *post-hoc* test for cholesterol showed significantly lower in CT + P (d: 2.05, $p < 0.001$), CT (d: 1.96, $p < 0.001$), S (d: 1.36, $p = 0.022$), and CT + S (d: 2.02, $p < 0.001$) compared with C group. *Post-hoc* test showed significantly lower in CT + P (d: 2.16, $p < 0.001$), CT (d: 2.03, $p < 0.001$), S (d: 1.46, $p = 0.009$), and CT + S (d: 2.13, $p < 0.001$) compared with P group. No difference was found between the other groups ($p > 0.05$) (Fig. 14).

Bonferroni's correction *post-hoc* test for TG showed significantly lower in CT + P (d: 2.81, $p < 0.001$), CT (d: 2.80, $p < 0.001$), S (d: 1.41, $p = 0.014$), and CT + S (d: 3.01, $p < 0.001$) compared with C group. *Post-hoc* test showed significantly lower in CT + P (d: 2.76, $p < 0.001$), CT (d: 2.75, $p < 0.001$), S (d: 1.36, $p = 0.021$), and CT + S (d: 2.95, $p < 0.001$) compared with P group. The TG were significantly higher in S compared to CT + P (d: − 1.40, $p = 0.020$)

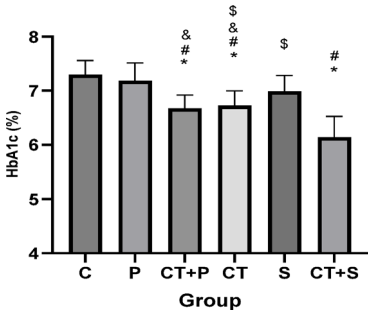

Fig. 13. Serum HbA1c level (Mean ± SD). * Indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. & indicates a significant difference with the S. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S* combined training + supplement.

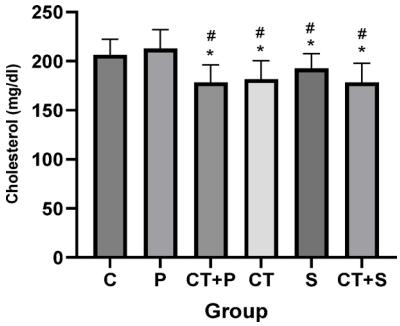

Fig. 14. Serum cholesterol level levels (Mean ± SD). * Indicates a significant difference with the C. # indicates a significant difference with the P. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S*combined training + supplement.

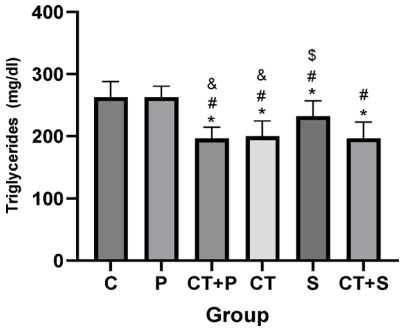

Fig. 15. Serum triglycerides level (Mean ± SD). * Indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. & indicates a significant difference with the S. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S* combined training + supplement.

and CT (d: − 1.38, $p = 0.018$) groups. Also, the *Post-hoc* test showed significantly lower in CT + S compared with S (d: 1.59, $p = 0.003$). No difference was found between the other groups ($p > 0.05$) (Fig. 15).

Bonferroni's correction *post-hoc* test for HDL showed significantly higher in CT + P (d: − 2.64, $p < 0.001$), CT (d: − 2.74, $p < 0.001$), S (d: − 1.31, $p = 0.032$), and CT + S (d: − 2.83, $p < 0.001$) compared with C group. The *Post-hoc* test showed significantly higher in CT + P (d: − 2.71, $p < 0.001$), CT (d: − 2.80, $p < 0.001$), S (d: − 1.37, $p = 0.021$), and CT + S (d: − 2.89, $p < 0.001$) compared with P group. The *Post-hoc* test showed significantly lower in S compared to CT + P (d: 1.33, $p = 0.026$) and CT (d: 1.43, $p = 0.013$). Also, HDL were significantly higher in CT + S compared with S group (d: − 1.52, $p = 0.006$). No difference was found between the other groups ($p > 0.05$) (Fig. 16).

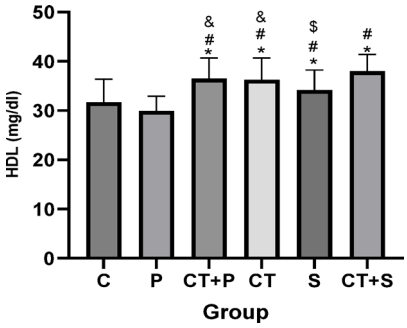

Fig. 16. Serum HDL level (Mean ± SD). * Indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. & indicates a significant difference with the S. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S* combined training + supplement.

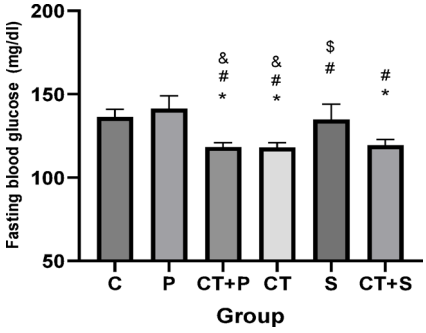

Fig. 17. Serum Fasting Blood Glucose level (Mean ± SD). * Indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. & indicates a significant difference with the S. *C* control group, *P* placebo, *CT* + *P* combined training + placebo, *CT* combined training, *S* supplement, and *CT* + *S* combined training + supplement.

Bonferroni's *post-hoc* correction test for Fasting Blood Glucose showed significantly lower in CT + P (d: 3.44, $p < 0.001$), CT (d: 3.50, $p < 0.001$), and CT + S (d: 3.29, $p < 0.001$) compared with C group. The *Post-hoc* test showed significantly lower in CT + P (d: 4.21, $p < 0.001$), CT (d: 4.27, $p < 0.001$), S (d: 1.25, $p = 0.047$), and CT + S (d: 4.05, $p < 0.001$) compared with P group. Also, the *Post-hoc* test showed significantly higher in S compared with CT + P (d: − 2.95, $p = 0.030$) and CT (d: − 3.01, $p = 0.030$). Also, the FBG were significantly lower in CT + S compared with S group (d: 2.81, $p < 0.001$). No difference was found between the other groups ($p > 0.05$) (Fig. 17).

Bonferroni's *post-hoc* correction test for Fasting insulin showed significantly lower in CT + P (d: 2.24, $p < 0.001$), CT (d: 2.73, $p < 0.001$), and CT + S (d: 2.20, $p < 0.001$) compared with C group. The *Post-hoc* test showed significantly lower in CT + P (d: 1.95, $p < 0.001$), CT (d: 1.06, $p = 0.045$), and CT + S (d: 1.56, $p = 0.005$) compared with P group. Also, Fasting insulin showed significantly higher in S compared with CT + P (d: − 2.95, $p = 0.030$) and CT (d: − 3.01, $p = 0.030$) groups. No difference was found between the other groups ($p > 0.05$) (Fig. 18).

Discussion

The aim of this study was to investigate whether HN and miRNAs-122, -126-3p, and -146a could mediate the reduction of OS and IF induced by CT and AST supplementation in women with T2DM, potentially leading to improved diabetic symptoms and complications. Our results showed that CT and AST supplementation improved antioxidant defense and their combination were more effective than either intervention alone. CT and AST supplementation also reduced IF, with the combination again being more effective. CT and AST supplementation also increase blood concentration of HN, and their combination showed greater effects than AST supplementation, but not CT. In addition, CT and AST supplementation increased blood levels of miRNAs-126-3p, and -146a and decreased miRNA-122 with their combination being slightly more effective in decreasing miRNA-122. Both interventions also improved lipid profile, with their combination being more effective in improving HDL and TG levels, although not cholesterol. Furthermore, FBG, IR, and HbA1c were reduced by CT but not by AST supplementation (Fig. 19).

Elevated OS can lead to cellular damage and dysfunction, which exacerbates IR and contributes to the development of diabetic complications. OS could also activates inflammatory pathways, resulting in chronic IF that further disrupts normal metabolic processes and accelerates IR[5,44]. Our results showed that while

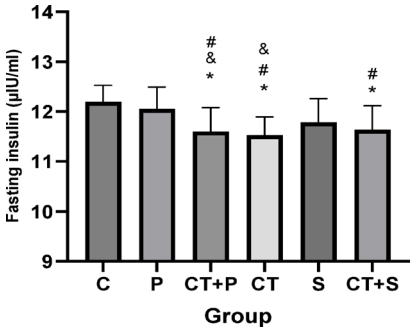

Fig. 18. Serum fasting insulin level (Mean ± SD). * Indicates a significant difference with the C. # indicates a significant difference with the P. $ indicates a significant difference with the CT + S. & indicates a significant difference with the S. *C* control group, *P* placebo, *CT + P* combined training + placebo, *CT* combined training, *S* supplement, and *CT + S* combined training + supplement.

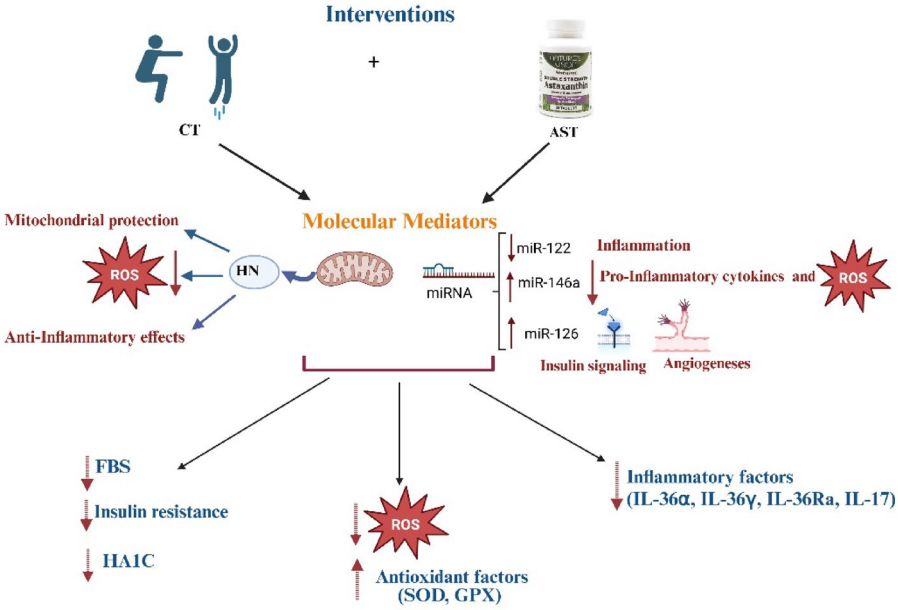

Fig. 19. Effect of - exercise and Astaxanthin supplementation on redox-sensetive miRNAs, Humanin, glucose metabolism oxidative stress and inflammation in type 2 diabetes .

TAC and serum levels of GPx were increased by both CT and AST supplementation, their combination led to more significant increases. Serum levels of SOD increased only with CT. Other studies also showed improved antioxidant defense in patients with T2DM following resistance training[45,46], endurance training[47,48]high-intensity interval training (HIIT)[45], TRX training[46], and acute exercise[49]. Xu et al. reported that treating rats with type 1 diabetes with 10, 20, and 40 mg/kg AST for 5 days increased levels of GSH and SOD while reducing MDA, with the highest effect seen at 40 mg/kg[50]. Other studies also confirmed the antioxidant effects of AST in diabetic patients[21,51,52]. In addition, a systematic review and meta-analysis, published recently, concluded that AST supplementation could lead to increase TAC in athletes and improve cycling time trail performance[53].

Effect of CT and AST on OS, IF and HN

Reducing OS could also relieve IF, as confirmed by our results. Both interventions reduced the levels of pro-inflammatory markers (e.g., IL-36α and IL-36γ) and increased the levels of anti-inflammatory markers (e.g., IL-36 Ra). Given that both aerobic and resistance training individually lead to beneficial changes in inflammatory markers in individuals with T2DM and/or obesity, combining them seems to be the most effective exercise strategy for those with metabolic health issues[12]. The integration of aerobic and resistance exercise elicits potent anti-inflammatory effects by modulating adipose tissue metabolism and reducing pro-inflammatory cytokines[13]. A recently published systematic review and meta-analysis by Khalafi et al,. concluded that CT is superior to resistance training and HIIT in reducing systemic IF in T2D patients[54]. Papagianni et al. showed a significant improvement in IF after aerobic training[10]. Decreased serum levels of IL-17 after 8 weeks of aerobic training (Burce protocol) in men with T2DM has been reported by Alikhazaeil et al.[55]. However, another study[56]

did not find an anti-inflammatory effect after concurrent training in sedentary obese participants. Differences in participant characteristics (obese in their study vs. T2DM patients in ours) and variations in exercise protocols could possibly explain this controversy. Other studies[57,58] aligned with our findings reported the anti-inflammatory effects of AST in diabetes. Chang et al.[20] highlighted the pathways that might be involved in anti-inflammatory effect of AST including PI3K/AKT, Nrf2, NF-κB, ERK1/2, JNK, p38 MAPK, and JAK-2/STAT-3.

Moin et al. reported that HN exhibited antioxidant and anti-inflammatory effects in diabetic rats[59]. HN administration could improve several diabetes symptoms including HbA1c levels, FBG, IR, and lipid profiles. Physiologically, HN is produced in various organs such as the kidneys, skeletal muscles, brain, heart, and liver, and is then secreted into the bloodstream[22]. It protects cells against diseases strongly linked to OS and mitochondrial dysfunction[22]. Exercise is a source of physiological stressor of mitochondria stimulating adaptive response such as HN[60]. Resistance training (three times per week for twelve weeks with progressively increasing intensity) raised serum HN levels in men with impaired glucose metabolism, ultimately improving IR[61]. HIIT[11] and endurance training[26] have also been shown to increase HN serum levels. Studies showed that CT could be stronger stimulation than endurance and resistance training for HN secretion because it expose mitochondria to both endurance related and load related stress promoting maximal mitochondria adaptation and subsequently HN secretion[62]. Our results indicate that CT and ASX supplementation improved HN levels, with their combination leading to higher HN levels than AST alone, though not more than CT alone.

Effect of CT and AST on MiRNAs-122, 126-3p and -146a

MiRNA-122, primarily expressed in the liver, suppressed TNF-α and IL-6 in human liver organoid models[63]. Studies[64,65] have indicated that HIIT may serve as a non-pharmacological intervention to stimulate miRNA-122 secretion. However, Zolfi et al., showed that HIIT reduced MiRNA-122 in middle-aged men with metabolic syndrome[65]. These controversial results indicate that the effect of exercise on MiRNA-122 is complicated and context dependent. So, we chose CT trying to improve the consistency as it includes both kinds of exercise. We observed that miRNA-122 levels decreased in the CT, S, and CT + S groups, with the lowest levels in the CT + S group.

MiRNAs-126-3p and -146a, which are also involved in antioxidant defense, increased after CT and AST supplementation. The combination of these interventions led to higher levels of these miRNAs compared to AST alone, but not more than CT alone. MiRNA-126-3p is expressed exclusively in endothelial cells within capillaries and larger blood vessels[66] and regulates IF by targeting various transcripts[67]. Overexpression of miRNA-126-3p has been shown to reduce IF in diabetic patients[30]. While previous studies[36,38] have shown increased miRNA-126–3-p expression following aerobic training, resistance training alone is seems not to increase blood levels of miRNA-126-3p in diabetic patients[68]. The reason could be the main origin of miRNA-126–3-p which is endothelial cells. Because aerobic exercise increase blood flow and shear stress more than resistance exercise, it could provide stronger stimulation for miRNA-126–3-p secretion[68]. miRNA-146a, located on the long arm of chromosome 5[69], has been found to play a protective role against IF by targeting genes involved in the inflammatory process[32]. Cirilli et al.[70] found that high-intensity training downregulated miRNA-146a. However, other studies reported upregulation of miRNA-146a following resistance training[71] and after 8 weeks of combined resistance training and HIIT[72] in diabetic patients. These positive effects were observed with different exercise regimens (same day vs. different days)[72]. The variation in results may be attributed to differences in exercise protocols (aerobic training in Cirilli et al. vs. CT in Ghodrat et al. and our study).

The only study that explored the impact of AST on miRNAs in diabetes was conducted by Shokri-Mashhadi et al.[73]. They found that administering 8 mg/day of oral AST for 8 weeks reduced circulating levels of miRNA-146a but did not affect miRNA-126. It is possible that differences in participant sex (male and female participants in their study vs. only females in ours) may explain why they did not observe a significant effect of AST on miRNA-126, as men might require higher doses of AST to see significant changes.

Effect of CT and AST on lipid profile, IR, HbA1c and FBG

Our data suggest that combining exercise with AST supplementation might improve oxidative status and IF through mechanisms involving HN and miRNAs 122, 126-3p, and 146a. Alleviating OS and IF could, in turn, lead to improvements in lipid profiles (e.g., TG, and HDL), IR, and reductions in HbA1c and FBG, as observed in our study. Furthermore, the combined approach seems to be more effective at improving cholesterol and TG levels.

A recent review by Al-Mhanna et al.[13] concluded that CT likely enhances cardio metabolic health by improving insulin sensitivity, increasing muscle glucose uptake, and promoting favorable changes in body composition, including reduced adiposity. We also believe that mitigating OS and IF, potentially through HN and miRNAs, could contribute to these benefits. Different mechanisms can facilitate aerobic exercise effect of cardiometabolic health such as increase energy expenditure and promoting fat loos, enhancing lipoprotein lipase (LPL) activity, and improving IR. But other mechanisms mediate resistance effect of cardiometabolic health including increase basal metabolic rate due to increase muscle mass, direct reduction of cholesterol and LDL, enhances muscle glucose uptake and increase LPL activity[74]. We believe that CT could induce all of the aerobic and resistance effects.

CT has been shown to improve various glucose metabolism parameters, including HOMA-IR and FBG, in healthy adults[14]. Previous studies[12,75] are consistent with our findings, indicating that CT improves IR and reduces FBG. Additionally, the reduction in HbA1c resulting from CT has been confirmed by multiple studies[8,76].

Previous researches have also shown reductions in total cholesterol[72], LDL[72] and increases in HDL. Tai chi has also been shown to improve lipid profiles in patients with T2D[77]. Bassi et al.[78] reported a decrease in LDL/HDL ratios after exercise training. Variations in exercise protocols, participant characteristics, and disease status might explain why some studies did not find significant changes in certain variables.

Additionally, the positive effects of AST on HbA1c[79], HOMA-IR[17,51,79], FBG[17,51], and lipid profiles[17,51,79] have been confirmed by previous studies.

Conclusion

Our study demonstrates that CT, along with AST supplementation, effectively reduces OS and IF. These improvements are mediated through changes in miRNAs-122, -136-3p and -146a and HN. The observed reductions in OS and IF are associated with favorable alterations in lipid profiles, enhanced IR, and reductions in HbA1c and FBG. Importantly, the combined approach of exercise training and AST supplementation often yields superior benefits compared to each intervention alone, suggesting a synergistic effect that enhances overall metabolic health.

Limitations

This study only enrolled female participants to minimize inter-individual biological variability arising from sex differences in hormone regulation, fat distribution, and gene expression related to inflammation and oxidative stress.

Data availability

The datasets generated and/or analyzed during this study, including the codebook and analytic code, will be made available to qualified researchers upon reasonable request to the corresponding author(s).

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References

1. Oguntibeju, O. O. Type 2 diabetes mellitus, oxidative stress and inflammation: Examining the links. *Int. J. Physiol Pathophysiol. Pharmacol.* **11**(3), 45 (2019).
2. Goodarzi, M. T., Navidi, A. A., Rezaei, M. & Babahmadi-Rezaei, H. Oxidative damage to DNA and lipids: Correlation with protein glycation in patients with type 1 diabetes. *J. Clin. Lab. Anal.* **24**(2), 72–76 (2010).
3. Kawahito, S., Kitahata, H. & Oshita, S. Problems associated with glucose toxicity: role of hyperglycemia-induced oxidative stress. *World J. Gastroenterol. WJG* **15**(33), 4137 (2009).
4. Yuan, T. et al. New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. *Redox Biol.* **20**, 247–260 (2019).
5. Suzuki, K. Chronic inflammation as an immunological abnormality and effectiveness of exercise. *Biomolecules* **9**(6), 223 (2019).
6. Sharifi-Rad, M. et al. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Front. Physiol.* **11**, 694 (2020).
7. King, G. L. The role of inflammatory cytokines in diabetes and its complications. *J. Periodontol.* **79**, 1527–1534 (2008).
8. Sabouri, M., Hatami, E., Pournemati, P. & Shabkhiz, F. Inflammatory, antioxidant and glycemic status to different mode of high-intensity training in type 2 diabetes mellitus. *Mol. Biol. Rep.* **48**, 5291–5304 (2021).
9. Beni, M. A., Rad, S. S. & Chamani, N. Comparison of the effects of eight weeks of traditional resistance training and TRX on oxidative and antioxidant indicators in women with type 2 diabetes. *J. Sport Exercise Physiol. (JSEP)/ Fīziyuluzhī-i Varzish va Faāliyyat-i Badanī* **16**(3), 66–75 (2023).
10. Papagianni, G. et al. The anti-inflammatory effects of aerobic exercise training in patients with type 2 diabetes: A systematic review and meta-analysis. *Cytokine* **164**, 156157 (2023).
11. Woodhead, J. S. et al. High-intensity interval exercise increases humanin, a mitochondrial encoded peptide, in the plasma and muscle of men. *J. Appl. Physiol.* **128**(5), 1346–1354 (2020).
12. Batrakoulis, A. et al. Comparative efficacy of 5 exercise types on cardiometabolic health in overweight and obese adults: A systematic review and network meta-analysis of 81 randomized controlled trials. *Circ. Cardiovasc. Qual. Outcomes.* **15**(6), e008243 (2022).
13. Al-Mhanna, S. B. et al. Effects of combined aerobic and resistance training on glycemic control, blood pressure, inflammation, cardiorespiratory fitness and quality of life in patients with type 2 diabetes and overweight/obesity: A systematic review and meta-analysis. *PeerJ* **12**, e17525 (2024).
14. Silva, F. M., Duarte-Mendes, P., Teixeira, A. M., Soares, C. M. & Ferreira, J. P. The effects of combined exercise training on glucose metabolism and inflammatory markers in sedentary adults: A systematic review and meta-analysis. *Sci. Rep.* **14**(1), 1936 (2024).
15. Jin, C.-H., Rhyu, H.-S. & Kim, J. Y. The effects of combined aerobic and resistance training on inflammatory markers in obese men. *J. Exercise Rehabil.* **14**(4), 660 (2018).
16. Tayebi, S. M. et al. Effects of swimming training in hot and cold temperatures combined with cinnamon supplementation on HbA1C levels, TBC1D1, and TBC1D4 in diabetic rats. *Nutr. Diab.* **14**(1), 1 (2024).
17. Ciaraldi, T. P. et al. Astaxanthin, a natural antioxidant, lowers cholesterol and markers of cardiovascular risk in individuals with prediabetes and dyslipidaemia. *Diabetes Obes. Metab.* **25**(7), 1985–1994 (2023).
18. Jabarpour, M., Aleyasin, A., Shabani Nashtaei, M. & Amidi, F. Astaxanthin supplementation impact on insulin resistance, lipid profile, blood pressure, and oxidative stress in polycystic ovary syndrome patients: A triple-blind randomized clinical trial. *Phytother. Res.* **38**(1), 321–330 (2024).
19. Nie, W., Li, J. & Rajabi, S. Molecular mechanisms, endurance athlete, and synergistic therapeutic effects of marine-derived antioxidant astaxanthin supplementation and exercise in cancer, metabolic diseases, and healthy individuals. *Food Sci. Nutr.* **13**(7), e70470 (2025).
20. Chang, M. X. & Xiong, F. Astaxanthin and its effects in inflammatory responses and inflammation-associated diseases: Recent advances and future directions. *Molecules* **25**(22), 5342 (2020).
21. Ma, B. et al. Astaxanthin supplementation mildly reduced oxidative stress and inflammation biomarkers: A systematic review and meta-analysis of randomized controlled trials. *Nutr. Res.* **99**, 40–50 (2022).
22. Cai, H., Liu, Y., Men, H. & Zheng, Y. Protective mechanism of humanin against oxidative stress in aging-related cardiovascular diseases. *Front. Endocrinol.* **12**, 683151 (2021).
23. Klein, L. E., Cui, L., Gong, Z., Su, K. & Muzumdar, R. A humanin analog decreases oxidative stress and preserves mitochondrial integrity in cardiac myoblasts. *Biochem. Biophys. Res. Commun.* **440**(2), 197–203 (2013).
24. Zhao, S.-T., Zhao, L. & Li, J.-H. Neuroprotective peptide humanin inhibits inflammatory response in astrocytes induced by lipopolysaccharide. *Neurochem. Res.* **38**, 581–588 (2013).

25. Yen, K. et al. The mitochondrial derived peptide humanin is a regulator of lifespan and healthspan. *Aging (Albany NY).* **12**(12), 11185 (2020).
26. von Walden, F. et al. Acute endurance exercise stimulates circulating levels of mitochondrial-derived peptides in humans. *J. Appl. Physiol.* **131**(3), 1035–1042 (2021).
27. Woodhead, J. S. & Merry, T. L. Mitochondrial-derived peptides and exercise. *Biochimica et Biophys. Acta Gen. Subj.* **1865**, 130011 (2021).
28. Soltany, A., Daryanoosh, F., Gholampour, F., Sadat Hosseini, N. & Khoramipour, K. Potential role of high-intensity interval training-induced increase in humanin levels for the management of type 2 diabetes. *J. Cell Mol. Med.* **29**(3), e70396 (2025).
29. Sawada, S. et al. Profiling of circulating microRNAs after a bout of acute resistance exercise in humans. *PLoS ONE* **8**(7), e70823 (2013).
30. Zeinali, F. et al. Circulating microRNA-122, microRNA-126-3p and microRNA-146a are associated with inflammation in patients with pre-diabetes and type 2 diabetes mellitus: A case control study. *PLoS ONE* **16**(6), e0251697 (2021).
31. Grieco, G. E. et al. Targeting microRNAs as a therapeutic strategy to reduce oxidative stress in diabetes. *Int. J. Mol. Sci.* **20**(24), 6358 (2019).
32. Bhatt, K. et al. Anti-inflammatory role of microRNA-146a in the pathogenesis of diabetic nephropathy. *J. Am. Soc. Nephrol.* **27**(8), 2277–2288 (2016).
33. Wan, R. J. & Li, Y. H. MicroRNA-146a/NAPDH oxidase4 decreases reactive oxygen species generation and inflammation in a diabetic nephropathy model. *Mol. Med. Rep.* **17**(3), 4759–4766 (2018).
34. Wang, J., Dong, Z., Lou, L., Yang, L. & Qiu, J. MiR-122 participates in oxidative stress and apoptosis in STZ-induced pancreatic β cells by regulating PI3K/AKT signaling pathway. *Int. J. Endocrinol.* **2021**(1), 5525112 (2021).
35. Kazemi, S. S. et al. Circulating levels of microRNA-122 and weight change in response to combined exercise interventions in metabolic syndrome. *Muscles Ligaments Tendons J. (MLTJ).* **15**(1), 107–112 (2025).
36. Jin, D., Yang, X.-Y. & Wang, J.-S. MicroRNA-126 level increases during exercise rehabilitation of heart failure with a preserved ejection fraction. *Int. J. Gen. Med.* **14**, 3397–3404 (2021).
37. Barber, J. L. et al. The effects of regular exercise on circulating cardiovascular-related microRNAs. *Sci. Rep.* **9**(1), 7527 (2019).
38. Riedel, S. et al. Exercise training improves high-density lipoprotein-mediated transcription of proangiogenic microRNA in endothelial cells. *Eur. J. Prev. Cardiol.* **22**(7), 899–903 (2015).
39. Klika, B. & Jordan, C. High-intensity circuit training using body weight: Maximum results with minimal investment. *ACSM's Health Fitness J.* **17**(3), 8–13 (2013).
40. Myers, T. R., Schneider, M. G., Schmale, M. S. & Hazell, T. J. Whole-body aerobic resistance training circuit improves aerobic fitness and muscle strength in sedentary young females. *J. Strength Cond. Res.* **29**(6), 1592–1600 (2015).
41. Story, C. et al. Comparison of methods for determining aerobic exercise intensity using heart rate in acute leukemia patients prior to induction chemotherapy. *Biol. Res. Nurs.* **18**(4), 432–438 (2016).
42. Ambelu, T. & Teferi, G. The impact of exercise modalities on blood glucose, blood pressure and body composition in patients with type 2 diabetes mellitus. *BMC Sports Sci. Med. Rehabil.* **15**(1), 153 (2023).
43. Alberti, K. G. M. M., Zimmet, P. & Shaw, J. Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. *Diab. Med.* **23**(5), 469–480 (2006).
44. Yousef, H., Khandoker, A. H., Feng, S. F., Helf, C. & Jelinek, H. F. Inflammation, oxidative stress and mitochondrial dysfunction in the progression of type II diabetes mellitus with coexisting hypertension. *Front. Endocrinol.* **14**, 1173402 (2023).
45. Sudarsono, N. C. et al. The effects of combined high-intensity interval and resistance training on glycemic control and oxidative stress in T2DM. *Asian J Sports Med* **10**(10), e91841 (2019).
46. Akbarpour, M., Sabagheyan Rad, S. & Chamani, N. Comparison of the effects of eight weeks of traditional resistance training and TRX on oxidative and antioxidant indicators in women with type 2 diabetes. *J. Sport Exercise Physiol.* **16**(3), 66–75 (2023).
47. Pittaluga, M. et al. Physical exercise and redox balance in type 2 diabetics: Effects of moderate training on biomarkers of oxidative stress and DNA damage evaluated through comet assay. *Oxid. Med. Cell Longev.* **2015**, 981242. https://doi.org/10.1155/2015/981242 (2015).
48. Ghasemi Pour, S. & Marandi, S. M. Effects of endurance training and chlorogenic acid on oxidative and nitrosative stress markers in prediabetic male mice. *Int. J. Prev. Med.* **16**, 5. https://doi.org/10.4103/ijpvm.ijpvm_122_23 (2025).
49. Kostic, N. et al. Clinical evaluation of oxidative stress in patients with diabetes mellitus type II–impact of acute exercise. *Vojnosanit Pregl.* **66**(6), 459–464 (2009).
50. Xu, L., Zhu, J., Yin, W. & Ding, X. Astaxanthin improves cognitive deficits from oxidative stress, nitric oxide synthase and inflammation through upregulation of PI3K/Akt in diabetes rat. *Int. J. Clin. Exp. Pathol.* **8**(6), 6083 (2015).
51. Choi, H. D., Youn, Y. K. & Shin, W. G. Positive effects of astaxanthin on lipid profiles and oxidative stress in overweight subjects. *Plant Foods Hum. Nutr.* **66**, 363–369 (2011).
52. Shokri-Mashhadi, N., Tahmasebi, M., Mohammadi-Asl, J., Zakerkish, M. & Mohammadshahi, M. The antioxidant and anti-inflammatory effects of astaxanthin supplementation on the expression of miR-146a and miR-126 in patients with type 2 diabetes mellitus: A randomised, double-blind, placebo-controlled clinical trial. *Int. J. Clin. Pract.* **75**(5), e14022. https://doi.org/10.1111/ijcp.14022 (2021).
53. Hasani, M., Arabpour, Z., Hasani, M., Saeedi, A. & Khodabakhshi, A. Effect of astaxanthin on physical activity factors, lipid profile, inflammatory markers, and antioxidants indices in athletic men: A systematic review and meta-analysis. *J. Funct. Foods* **122**, 106477 (2024).
54. Khalafi, M. et al. Comparative efficacy of different exercise modes on inflammatory markers in patients with type 2 diabetes mellitus: A systematic review with pairwise and network meta-analyses. *Obes. Rev.* **26**, e13954. https://doi.org/10.1111/obr.13954 (2025).
55. AliKhazaeil, H. et al. The effect of 8 weeks aerobic training on serum levels of pro-inflammatory cytokines (IL-17) and immunoglobulins (IgA, IgM, IgG and IgE) levels in patients with type 2 diabetes. *Ann. Med. Health Sci. Res.* **8**(6), 376–379 (2018).
56. Amaro-Gahete, F. J. et al. Effect of a 12-week concurrent training intervention on cardiometabolic health in obese men: A pilot study. *Front. Physiol.* **12**, 630831. https://doi.org/10.3389/fphys.2021.630831 (2021).
57. Yeh, P.-T., Huang, H.-W., Yang, C.-M., Yang, W.-S. & Yang, C.-H. Astaxanthin inhibits expression of retinal oxidative stress and inflammatory mediators in streptozotocin-induced diabetic rats. *PLoS ONE* **11**(1), e0146438 (2016).
58. Kim, B. et al. Astaxanthin inhibits inflammation and fibrosis in the liver and adipose tissue of mouse models of diet-induced obesity and nonalcoholic steatohepatitis. *J. Nutr. Biochem.* **43**, 27–35 (2017).
59. Moin, H. et al. Effectiveness of analog of Humanin in ameliorating streptozotocin-induced diabetic nephropathy in Sprague Dawley rats. *Peptides* **165**, 171014 (2023).
60. Gidlund, E. K. et al. Humanin skeletal muscle protein levels increase after resistance training in men with impaired glucose metabolism. *Physiol. Rep.* **4**(23), e13063. https://doi.org/10.14814/phy2.13063 (2016).
61. von Walden, F. et al. Humanin skeletal muscle protein levels increase after resistance training in men with impaired glucose metabolism. *FASEB J.* **31**, 1020 (2017).
62. Woodhead, J. S. T. & Merry, T. L. 2021 Mitochondrial-derived peptides and exercise. *Biochim. Biophys. Acta Gen. Subj.* **12**, 130011 (1865).
63. Sendi, H. et al. miR-122 inhibition in a human liver organoid model leads to liver inflammation, necrosis, steatofibrosis and dysregulated insulin signaling. *PLoS ONE* **13**(7), e0200847 (2018).

64. Kalaki-Jouybari, F., Shanaki, M., Delfan, M., Gorgani-Firouzjae, S. & Khakdan, S. High-intensity interval training (HIIT) alleviated NAFLD feature via miR-122 induction in liver of high-fat high-fructose diet induced diabetic rats. *Arch. Physiol. Biochem.* **126**(3), 242–249 (2020).
65. Zolfi, H. R., Shakib, A. & Niknam, Z. Impact of high-intensity interval training on Mir-21 and Mir-122 expression and metabolic parameters in middle-aged men with metabolic syndrome. *Jorjani Biomed. J.* **11**(3), 25–29 (2023).
66. van Solingen, C. et al. Antagomir-mediated silencing of endothelial cell specific microRNA-126 impairs ischemia-induced angiogenesis. *J. Cell Mol. Med.* **13**(8a), 1577–1585 (2009).
67. Lou, Z., Li, Q., Wang, C. & Li, Y. The effects of microRNA-126 reduced inflammation and apoptosis of diabetic nephropathy through PI3K/AKT signalling pathway by VEGF. *Arch. Physiol. Biochem.* **128**(5), 1265–1274 (2022).
68. Ma, Y. et al. Roles of physical exercise-induced MiR-126 in cardiovascular health of type 2 diabetes. *Diabetol. Metab. Syndr.* **14**(1), 169. https://doi.org/10.1186/s13098-022-00942-6 (2022).
69. Abou-Zeid, A., Saad, M. & Soliman, E. MicroRNA 146a expression in rheumatoid arthritis: association with tumor necrosis factor–alpha and disease activity. *Genet. Test. Mol. Biomarkers* **15**(11), 807–812 (2011).
70. Cirilli, I. et al. Three months monitored metabolic fitness modulates cardiovascular risk factors in diabetic patients. *Diabetes Metab. J.* **43**(6), 893–897 (2019).
71. Morais Junior, G. S. et al. Acute strength training promotes responses in whole blood circulating levels of miR-146a among older adults with type 2 diabetes mellitus. *Clin. Interv. Aging.* **12**, 1443–1450 (2017).
72. Ghodrat, L., Razeghian Jahromi, I., Koushkie Jahromi, M. & Nemati, J. Effect of performing high-intensity interval training and resistance training on the same day vs. different days in women with type 2 diabetes. *Eur. J. Appl. Physiol.* **122**(9), 2037–2047 (2022).
73. Shokri-Mashhadi, N., Mohammadshahi, M., Samandari, S. & Saadat, S. The effect of astaxanthin supplementation on cognitive function and depression in patients with type 2 diabetes: A double-blind, randomised controlled trial. *J. Health Syst. Res.* **15**(4), 304–309 (2020).
74. Beqa Ahmeti, G., Idrizovic, K., Elezi, A., Zenic, N. & Ostojic, L. Endurance training vs. circuit resistance training: Effects on lipid profile and anthropometric/body composition status in healthy young adult women. *Int. J. Environ. Res. Public Health.* **17**(4), 1222. https://doi.org/10.3390/ijerph17041222 (2020).
75. Batrakoulis, A. et al. Hybrid neuromuscular training improves cardiometabolic health and alters redox status in inactive overweight and obese women: A randomized controlled trial. *Antioxidants.* **10**(10), 1601 (2021).
76. Jorge, M. L. M. P. et al. The effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with type 2 diabetes mellitus. *Metabolism* **60**(9), 1244–1252 (2011).
77. Zhang, F. et al. Metabolic impairments associated with type 2 diabetes mellitus and the potential effects of exercise therapy: An exploratory randomized trial based on untargeted metabolomics. *PLoS ONE* **19**(3), e0300593 (2024).
78. Bassi, D. et al. Potential effects on cardiorespiratory and metabolic status after a concurrent strength and endurance training program in diabetes patients—a randomized controlled trial. *Sports Med. Open.* **2**, 1–13 (2016).
79. Urakaze, M. et al. The beneficial effects of astaxanthin on glucose metabolism and modified low-density lipoprotein in healthy volunteers and subjects with prediabetes. *Nutrients* **13**(12), 4381 (2021).

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Author contributions
K.KH, A.B and Ka.KH: Conceptualization, Methodology, Writing—original draft, Writing—review & editing. A.B: Data Analysis, Writing—original draft, review an editing. N.H, A.KH, M.HH and L.A: Writing—review and editing.

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Declarations

Competing interests
The authors declare no competing interests.

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