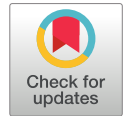








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Preliminary Study: Physiological Responses to a Single Bout of Nordic Walking Exercise in Patients with Pre-hypertensive Post-menopausal Women



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Abstract

Objective: Cardiovascular diseases are a significant cause of morbidity and mortality globally, particularly among post-menopausal women who face heightened risk. Exercise is recognized for its preventive benefits on cardiovascular health and enhancement of vascular function; yet, the effects of various exercise modalities remain inadequately understood. This study aimed to assess alterations in erythrocyte deformability, total oxidant/antioxidant status (TOS/TAS), oxidative stress index (OSI), and serum levels of leptin and irisin in pre-hypertensive post-menopausal women following one session of Nordic walking (NW) exercise.

Materials and Methods: The study cohort comprised 11 post-menopausal women with pre-hypertension (mean age 57.91 ± 7.23 years) and 12 age-matched healthy women (mean age 55.17 ± 5.29 years). Venous blood was taken before and just after the exercise session. The deformability of red blood cells (RBCs) was assessed using an ektacytometer. TOS/TAS, irisin, and leptin were quantified using commercial assays.

Results: A session of NW exercise did not influence oxidative stress indices or blood levels of irisin and leptin; nevertheless, erythrocyte deformability assessed at 30.00 Pa significantly increased post-exercise ($p=0.043$).

Conclusion: Our findings indicate that enhanced RBC deformability after an acute session of NW may facilitate blood flow.

Keywords


Red blood cell deformability · Nordic walking exercise · Oxidative stress · Irisin and Leptin



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INTRODUCTION

Pre-hypertension is associated with cognitive decline, increased left ventricular mass, and arteriosclerosis (1). Currently, the advised intervention for pre-hypertension is lifestyle modification. Weight reduction, sodium limitation, increased physical activity, cessation of smoking, and abstinence from alcohol exemplify lifestyle modifications (2). Menopause correlates with an age-dependent increase in artery damage and a decrease in muscle strength, thereby increasing the risk of cardiovascular disease. Thus, reducing arterial damage and lowering blood pressure may be advantageous in decreasing the incidence of cardiovascular disease in post-menopausal women with pre-hypertension (3).

Exercise has been allowed to mitigate age-related cardiovascular disease risk factors. Nordic walking (NW) is an aerobic workout that has attained considerable popularity as a form of physical activity. In contrast to walking, it offers the benefit of using the upper body and arms, hence alleviating stress on the hips, knees, and feet (4).

Hemorheology is a scientific discipline that investigates the characteristics of blood flow and the interactions between blood and blood arteries. Deformability of red blood cells (RBCs) is essential for their function in oxygen delivery, as indicated by an elevated elongation index (EI) (5). Enhanced EI at a specific shear stress signifies increased cellular deformability. Articles on the impacts of exercise on RBC deformability show variability attributed to differences in exercise volume and intensity, studied population and the devices used to measure deformability (6). Diseases such as diabetes, hypercholesterolemia, and hypertension may lead to diminished RBC deformability because of heightened oxidative stress or reduced NO bioavailability (7).

The removal of free radicals by antioxidant enzymes and substances is often balanced by the production of free radicals. In the human body, oxidative pathway components can be quantified from biological samples; however, this procedure may be expensive and time-consuming. The assessment of the total oxidant status (TOS) and total antioxidant status (TAS), which reflect the cumulative and interaction influences of oxidant and antioxidant substances, provide a more thorough approach for evaluating the oxidant/antioxidant equilibrium. There are very few clinical studies investigating oxidative stress and the antioxidant system with NW exercise (8, 9).

Proteins and other cytokines secreted by the skeletal muscle are called "myokines." Myokines greatly enhance the contact between the muscle and adipose tissue, liver and pancreas.

Leptin and irisin are markers of glucose metabolism and control, and are linked to visceral obesity (10). Although no studies have examined the acute exercise response on irisin in post-menopausal women, research has examined the impact of acute exercise on leptin levels in this population (11, 12). The data mentioned above show that obesity and being overweight are significant problems for women after menopause. This study was conducted because of the considerable acceptance of physical activity through NW training (13). Research indicates that NW training, used as a body composition intervention, is most effective when supervised by trainers (14).

Nonetheless, the impact of NW on red blood cell deformability, TOS, TAS, oxidative stress index (OSI), and the levels of leptin and irisin has not yet been investigated in pre-hypertensive post-menopausal women. This study aimed to investigate the acute effects of a single session of NW exercise on erythrocyte deformability, oxidative stress markers (TOS/TAS, OSI), and serum levels of leptin and irisin in pre-hypertensive post-menopausal women.

MATERIALS AND METHODS

The work protocol adhered to the ethical principles established in the 1975 Declaration of Helsinki (ethical approval no: 60116787-020/5756). All participants received a comprehensive written and verbal description of the potential risks and advantages of participation.

Patients

In our study, patients exhibiting systolic blood pressure (SBP) ranging from 120 to 129 mmHg and diastolic blood pressure (DBP) below 80 mm Hg were classified as having high blood pressure (formerly known as pre-hypertension) (1). The exclusion criteria were as follows: SBP of 180 mmHg or more and/or DBP of 90 mmHg or more, current anti-hypertensive medications used, presence of rheumatoid arthritis, diabetes mellitus and inability to obtain approval from a primary care physician to participate in the study.

Twenty-three post-menopausal women with pre-hypertension (mean age 56.48 ± 6.30 years) participated in the study. Individuals who had not engaged in regular physical activity in the last six months were included in the study. Participants were randomly assigned to two groups: NW ($n=11$; mean age 57.91 ± 7.23 years) and control ($n=12$; mean age 55.17 ± 5.29 years). All participants were required to provide medical documentation confirming their eligibility for physical activity, and the groups were similar in terms of physical fitness and consistent with a sedentary lifestyle.



Anthropometric Assessments

Height in centimeters was measured using a stadiometer with the participant in the inspiratory phase in the anatomical position. This measurement was performed with an accuracy of ± 1 mm in centimeters.

On the first day of the study, body composition was assessed using a TANITA (BC-480) body composition analyzer while participants wearing lightweight shorts and a t-shirt. Participants were asked to wear the same attire for each subsequent measurement session. Special attention was given to ensure that all participants maintained a consistent posture throughout the measurements. To enhance the conductivity, the steel platform where the participants placed their feet was cleaned with a damp cloth before each measurement. Additionally, participants were instructed to fast for a minimum of 8 h before undergoing the assessments.

Aerobic Test: Prediction of Maximum Oxygen Uptake capacity (VO_{2max}) by Cooper 12-minute Run/Walk Test

The subjects spent 12 minutes running on a 400 m circular track. Their desire to complete as many laps as possible drove them. The finish line was marked, and the total number of laps was tallied. The VO_{2max} was predicted using the following equation once the distance in meters was translated to kilometers (15).

$$VO_{2max} = (22.351 \times \text{distance [km]}) \times 11.288$$

Sit and Reach Test

The sit-and-reach test was utilized to assess the flexibility of the lumbar extensors, hip extensors, gastrocnemius and hamstring muscles with a sit-and-reach test box. Participants, having removed their shoes, took their places on the bench, guaranteeing. Their toes were oriented upward. They were directed to extend the measuring ruler progressively by bending at the hips and extending forward maximally while maintaining fully extended legs. Participants were asked to maintain this maximum position for 2 s. The test was conducted thrice, and the highest score, measured in centimeters, was used for analysis (16).

Grip Strength Measurement

The hand grip strength was assessed using a calibrated digital Jamar dynamometer (Performance Health, Warrenville, IL). Participants were required to exert maximum force three times with each hand while maintaining the elbows at a 90-degree flexion and the wrists in a neutral posture. The optimal value was observed (17).

Exercise Protocol

Exercise sessions were conducted outdoors on a flat surface, beginning with a 10-to 12 min warm-up period. During the warm-up, each movement was performed in 4–6 repetitions to reduce muscle stiffness and maintain joint range of motion. Following the warm-up, the participants engaged in 50 min of NW, a full-body aerobic exercise performed with specially designed poles, involving the coordinated use of both the upper and lower extremity muscles. The walks were conducted during the spring, between 2:00 PM and 4:00 PM. Throughout the study, the weather conditions were characterized by moderate temperature levels. This technique includes not only forward walking but also technical aspects that require motor coordination and attention, such as correct pole placement, synchronization of arm and leg movements, maintaining appropriate stride length, and sustaining rhythmic movement throughout the activity. To ensure familiarity with the exercise, the participants were shown instructional videos and participated in a 5-min familiarization session before the walking session. The walks were conducted using a Polar M200 wrist-based heart rate (HR) tracking watch with GPS, which measures the HR. The single session NW program was conducted at a mean HR of 40–55% of the maximum age-related HR (18).

$$HR_{max} = 206 - 0.88 \times (\text{age})$$

$$[(HR_{max} - HR_{rest}) \times (0.4 \text{ to } 0.55)] + HR_{rest}$$

8–10 mL of venous blood was collected from the pre-hypertension group before and immediately after exercise and the control group only once.

Blood Samples

8–10 mL of venous blood was collected from the antecubital vein into tubes [EDTA (1.5 mg/mL)] from the pre-hypertension group before and immediately after exercise and the control group only once for analysis of complete blood count and hemorheological parameters. Venous blood samples (10 mL) were collected before the exercise, following an 8 h fasting period in the morning before any physical activity. Additionally, participants rested for at least 10 min in a seated position before blood collection. The samples were conveyed to the Physiology Laboratory in appropriate tubes and under proper transportation conditions, where hemorheological tests were performed within 3 h in accordance with the manual of hemorheological laboratory procedures (19). Blood samples (4–5 mL) were obtained in yellow top tubes containing gel (which includes a coagulation activator and separator) for the measurement of biochemical parameters, oxidative stress indicators, and serum levels of irisin and leptin. Blood was



centrifuged at 7260 rpm for 6 min, after which the serum was removed and preserved at -80°C .

Measurements of Erythrocyte Deformability

RBC deformability was assessed using laser diffraction analysis with an ektacytometer (Laser-Assisted Optical Rotational Cell Analyzer [LORCA], RR Mechatronics, Hoorn, The Netherlands). The system and methodology have been described in detail, previously (20). Briefly, a low hematocrit (Hct) suspension of RBCs in an isotonic, viscous medium (4% polyvinylpyrrolidone 360 solution; molecular weight 360 kDa; Sigma P5288, St. Louis, MO, USA) was subjected to shear stress within a Couette system comprising a glass cup and a rotating bob, creating a 0.3 mm gap. A laser beam was passed through the sheared suspension, and the resulting diffraction pattern was analyzed by a microcomputer. The EI was calculated using the formula $EI = (L - W)/(L + W)$, where L and W represent the major and minor axes of the elliptical diffraction pattern, respectively. The EI values were recorded at nine shear stress levels ranging from 0.3 to 30 Pa, revealing consistent deformability trends across the groups at all shear rates. All measurements were performed at 37°C (21, 22).

Assessment of the TOS/TAS and OSI

The serum TOS was measured using a fully automated, colorimetric method developed by Erel (23). In this assay, oxidants present in the serum oxidize the ferrous ion-O-dianisidine complex to the ferric ion, a reaction that is enhanced in the presence of glycerol. The resulting ferric ions formed a colored complex with xylenol orange in an acidic medium. The intensity of the color, measured spectrophotometrically, is directly proportional to the total amount of oxidant molecules (e.g., lipids, proteins) in the serum. The assay was calibrated using hydrogen peroxide, and the results were expressed in micromolar hydrogen peroxide equivalents per liter ($\mu\text{mol H}_2\text{O}_2$ equiv/L).

The serum TAS was evaluated using another automated colorimetric method developed by Erel (24). In this method, hydroxyl radicals generated by the Fenton reaction react with the colorless substrate O-dianisidine to form a bright yellowish-brown dansyl radical. Upon the addition of a serum sample, the antioxidant components inhibit this oxidative reaction, thereby preventing the formation of color. The degree of suppression reflects the serum's total antioxidant capacity. Results are expressed as millimoles of Trolox equivalent per liter (mmol Trolox/L).

Serum Exercise (irisin, leptin) Levels Assessment

Irisin and leptin levels were determined using ELISA Kits (Shanghai, YL Biotech Co.). According to the manufacturer's protocols, the commercial kits used were specific for measuring irisin and leptin in human samples. The detection limit was 5.0 pg/mL for the kits.

Statistical Analyses

A power analysis was performed based on the expected results. Accordingly, when at least 22 participants (at least 11 per group) were included in the study, that would result in 80% power with 95% confidence level (5% type 1 error rate) and the effect size was $d_z = 6.84$. Statistical analyses were performed using GraphPad Prism software, version 6 (GraphPad Software, La Jolla, CA, USA). One-way ANOVA and independent samples t test were applied to normally distributed data, while Kruskal-Wallis and Mann-Whitney U tests were used for non-normally distributed data. Statistical significance was defined as $p < 0.05$.

RESULTS

Effect of NW on Physiological and Clinical Assessment Variables

Physiological and clinical assessment variables such as anthropometric measurements, blood pressure characteristics, functional fitness components, biochemical data, and hematological parameters were all within normal ranges at the start of the study and did not show any statistically significant differences between the groups (Table 1-5). All of the participants completed the exercise protocol session without any complications.

The study enrolled 11 pre-hypertensive post-menopausal women (mean age was 57.91 ± 7.23 years) and 12 age-matched pre-hypertensive post-menopausal women controls (mean age was 55.17 ± 5.29 years).

Erythrocyte Deformability Values

RBC deformability was determined as EI. The differences between before and after the exercise regarding EI values were not statistically significant except at 30.00 Pa shear stress value in patients with pre-hypertension EI values measured at 30.00 Pa increased after the NW exercise ($p = 0.043$). The Hct value was increased after the exercise compared to before exercise ($p = 0.008$) (Table 6).

TOS/TAS and OSI Values

The TOS, TAS, and OSI of the participants in response to one session of the NW exercise and the control group are



Table 1. Anthropometric measurements of the participants

Parameters	Controls (n=12)	The NW exercise (n=11)	p
Age (years)	55.17 ± 5.29	57.91 ± 7.23	0.379
Body height (cm)	156.83 ± 3.1	155.82 ± 5.49	0.413
Body weight (kg)	73.73 ± 12.75	72.36 ± 11.47	0.566
BMI (kg/cm ²)	30.02 ± 4.69	29.68 ± 3.4	0.928
Percentage body fat (%)	38.65 ± 7.52	38.23 ± 5.58	0.601
Body muscle mass (kg)	42.32 ± 3.31	42.04 ± 3.99	1.000
Body fat mass (kg)	29.35 ± 9.94	28.14 ± 8.2	0.566
Total body water (kg)	32.63 ± 3.13	32.37 ± 3.13	1.000
Arm circumference (cm)	31.5 ± 3.49	34.8 ± 3.62	0.268
Forearm circumference (cm)	25.43 ± 2.52	27.3 ± 1.79	0.202
Wrist circumference (cm)	17.14 ± 1.07	17 ± 0.71	0.876
Chest circumference (cm)	105.43 ± 10.52	106.2 ± 6.72	1.000
Abdominal circumference (cm)	100.91 ± 14.74	100.72 ± 11.8	0.882
Hip circumference (cm)	112.09 ± 11.58	110.61 ± 8.56	0.941
Thigh circumference (cm)	56.57 ± 5.26	58.2 ± 7.76	0.876
Calf circumference (cm)	38 ± 3.86	39.4 ± 3.34	0.639
Ankle circumference (cm)	23.5 ± 3.12	23 ± 1.54	0.876

Values are expressed as mean ± standard deviation. NW: Nordic walking; BMI: Body mass index.

Table 2. Blood pressure characteristics of the participants

Parameters	Controls (n=12)	Before the NW exercise (n=11)	After the NW exercise (n=11)	p ₁	p ₂
Systolic pressure (mm/hg)	127.14 ± 17.04	133.81 ± 13.15	122 ± 12.29*	0.740	0.038
Diastolic pressure (mm/hg)	77.14 ± 9.51	79.54 ± 14.46	73 ± 8.23*	0.230	0.026

Values are expressed as mean ± standard deviation; NW: Nordic walking; p₁ value: difference with controls and before NW exercise; p₂ value: difference with before and after NW exercise value; *:p<0.05 statistically significant.

demonstrated in Figures 1A and 1B. Statistically insignificant changes were observed in the oxidative stress indices (p>0.05).

Serum Exercise (irisin and leptin) Levels

The serum irisin and leptin of the participants are demonstrated in Figure 1C. The acute effects of NW exercise caused no significant changes in irisin and leptin levels (p>0.05).

Table 3. Functional fitness components of the participants

Parameters	Controls (n=12)	The NW exercise (n=11)	p
Cooper 12-min run/walk test (m)	1310 ± 102.79	1236 ± 179.81	0.530
VO _{2max} (L/dk)	18.01 ± 2.31	16.36 ± 4	0.530
Sit and reach test (cm)	3.90 ± 7.02	2.61 ± 6.85	0.740
Grip strength (right) (bar)	22.18 ± 4.22	19.68 ± 6.32	0.402
Grip strength (left) (bar)	21.75 ± 3.64	18.55 ± 6.80	0.369

Values are expressed as mean ± standard deviation; NW: Nordic walking; VO_{2max}: Maximum oxygen uptake capacity.

Table 4. Biochemical data of the participants

Parameters	Controls (n=12)	The NW exercise (n=11)	p
Glucose (mmol/L)	105.89 ± 20.34	96 ± 10.73	0.243
Insulin (pmol/L)	12.13 ± 5.55	11.58 ± 4.65	0.720
HOMA-IR	3.27 ± 1.90	2.73 ± 1.28	0.549
Albumin (g/dL)	46.6 ± 2.59	45.5 ± 2.38	0.278
Cholesterol (mmol/L)	213.33 ± 36.79	209.8 ± 31.42	0.661
Triglycerides (mmol/L)	157.22 ± 71.49	107.6 ± 34.63	0.095
Creatine kinase (U/L)	79.38 ± 27	71.6 ± 24.44	0.515
Fibrinogen (mmol/L)	291.5 ± 37	334.7 ± 80.58	0.315
LDL (mmol/L)	120 ± 23.39	125.8 ± 31.74	0.549
ALP (U/L)	75.44 ± 19.79	69.9 ± 9.61	0.549
LDH (U/L)	180.33 ± 26.12	175.8 ± 16.27	0.780
Phosphor (mg/dL)	3.58 ± 0.45	3.75 ± 0.59	0.842
Magnesium (mg/mL)	2.11 ± 0.13	2.03 ± 0.14	0.243
HDL (mmol/L)	58.56 ± 17.59	62.5 ± 7.82	0.315
VLDL cholesterol (mmol/L)	31.33 ± 14.45	21.5 ± 6.92	0.095

Values are expressed as mean ± standard deviation; NW: Nordic walking; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; ALP: Alkaline phosphatase; VLDL: Very low-density lipoprotein; LDH: Lactate dehydrogenase.

DISCUSSION

NW is increasingly recognized as an effective form of physical activity, particularly among middle-aged and older adults in Central and Northern Europe. The positive effects of NW on cardiovascular health, aerobic capacity, and blood pressure regulation have been widely documented, given its potential to be integrated into daily routines and its suitability for women (13, 14). This study aimed to investigate the acute effects of a single session of NW on selected physiological and biochemical markers in post-menopausal women with prehypertension.



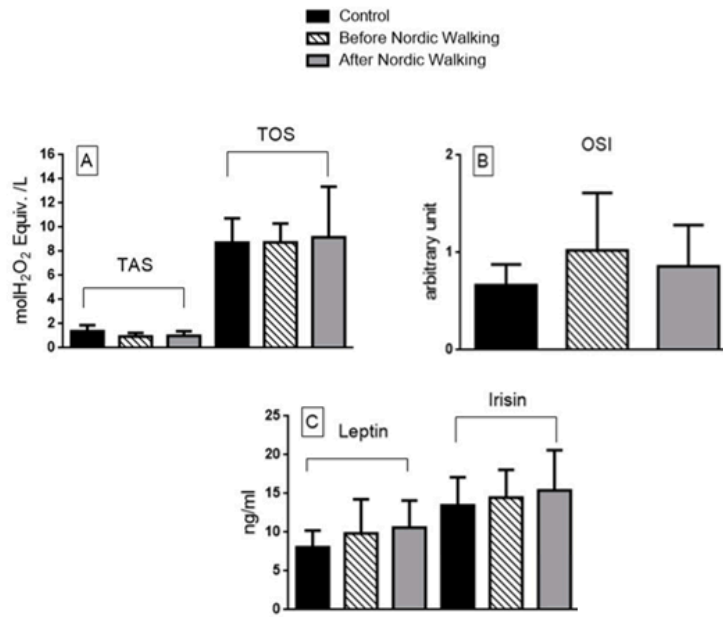


Figure 1. Total antioxidant status (TAS) and total oxidant status (TOS) values of the participants. B. Oxidative stress index (OSI) values of the participants. C. Irisin and leptin levels values of the participants. Mean ± standard deviation are used to express values.

Table 5. Whole blood count analyses of the participants

Parameters	Controls (n=12)	The NW exercise (n=11)	p
WBC (k/uL)	7.08 ± 1.79	6.89 ± 1.26	0.968
NEU (%)	58.66 ± 7.21	46.23 ± 16.36	0.053
LYM (%)	31.67 ± 7	39.04 ± 9.41	0.079
MONO (%)	5.94 ± 1.36	5.95 ± 1.24	0.968
BASO (%)	0.53 ± 0.22	0.44 ± 0.23	0.400
EO (%)	3.2 ± 1.66	2.86 ± 2.89	0.182
Hb (gm/dL)	14.1 ± 0.86	13.9 ± 0.67	0.549
MCV (fL)	89.77 ± 2.62	90.35 ± 3.79	0.842
MCH (pg)	29.81 ± 0.93	29.76 ± 1.19	0.968
MCHC (g/dL)	33.21 ± 0.69	32.94 ± 0.81	0.661
RDW (%)	13.04 ± 0.71	13.26 ± 0.6	0.720
PLT (k/uL)	260.44 ± 74.62	272.6 ± 72.59	0.780
PCT (ng/mL)	0.25 ± 0.05	0.24 ± 0.06	0.842

Mean ± standard deviation are used to express values. WBC: White blood cell; NEU: neutrophil; LYM: Lymphocyte; MONO: Monocyte; BASO: Basophil; EO: Eosinophil; Hb: Hemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red blood cell distribution width; PLT: Platelet count; PCT: Procalcitonin.

The findings of this study indicate that a single session of NW did not lead to significant changes in oxidative stress indices, serum irisin, and leptin levels in pre-hypertensive post-menopausal women. Additionally, no significant differences were found between the groups in physical fitness

Table 6. The participants' EI values under various shear stresses

Shear stress (Pa)	The EI values of the participants at different shear stresses		
	Before the NW exercise (n=11)	After the NW exercise (n=11)	p
0.30	0.061 ± 0.02	0.059 ± 0.01	0.646
0.53	0.125 ± 0.03	0.125 ± 0.02	0.533
0.95	0.222 ± 0.03	0.225 ± 0.03	0.799
1.69	0.562 ± 0.03	0.563 ± 0.02	0.878
3.00	0.425 ± 0.02	0.430 ± 0.02	0.625
5.33	0.503 ± 0.01	0.506 ± 0.01	0.449
9.49	0.562 ± 0.01	0.563 ± 0.01	0.929
16.87	0.603 ± 0.01	0.605 ± 0.01	0.385
30.00	0.629 ± 0.01	0.633 ± 0.01	0.043*
Hct (%)	47.10 ± 3.30	49.64 ± 3.32	0.0086*

Mean ± standard deviation are used to express values. NW: Nordic walking; Hct: Hematocrit; * p-value < 0.05 statistically significant.

components such as flexibility, strength, and aerobic capacity, which implies that the participants had similar physical fitness characteristics. The participants' VO_{2max} values suggest that post-menopausal women generally have low aerobic capacity, which is consistent with a sedentary lifestyle (25). This may have implications for their metabolic and vascular responses to exercise, potentially blunting the acute biochemical adaptations expected from physical activity.



In terms of blood pressure, we noted a significant reduction post-exercise, which aligns with the well-documented phenomenon of post-exercise hypotension (PEH). The lack of knowledge about the regulation of hypertension by physical exercise has resulted in contradictory findings; for example, acute aerobic exercise decreases resting blood pressure by 5–7 mmHg in individuals with hypertension after a single session, with effects that are immediate and can persist for up to 24 h in ambulatory situations during daytime (26). Although the mechanisms underlying PEH are multifactorial, they likely involve alterations in autonomic regulation, vascular resistance, and endothelial function. On the other hand, long-term aerobic exercise training reduces resting blood pressure more in individuals with hypertension (27). Similarly, Latosik et al. evaluated the effects of an 8-week supervised NW program on systolic blood pressure in postmenopausal women with systolic hypertension, and their findings demonstrated a significant reduction in SBP, along with improvements in both lower and upper body strength among participants who engaged in the NW training. The 8-week NW regimen may effectively reduce systolic hypertension by directly lowering SBP and enhancing maximal aerobic capacity (28). Consistent with the existing literature, our findings contribute to the growing body of evidence suggesting that even a single session of NW may induce favorable cardiovascular responses in pre-hypertensive individuals. However, to fully elucidate the underlying mechanisms, further long-term studies with larger sample sizes are warranted.

Our results indicate that a single NW session led to statistically significant improvements in erythrocyte deformability at high shear stress (30.00 Pa) and Hct levels. On the other hand, erythrocyte deformability was measured at 9 different shear stresses between 0.3 and 30 Pa, representing the shear stress levels that blood encounters at different regions of circulation. It is known that erythrocyte deformability is affected by membrane skeleton elasticity, cytoplasmic viscosity and cell geometry (surface-volume ratio), and polyunsaturated fatty acids play a key role in the structure and functioning of cell membranes (6, 7). To summarize, erythrocyte deformability can be affected in the circulatory system through a variety of mechanisms. Our findings suggest that even a single moderate-intensity bout of NW may positively influence the hemorheological properties and blood oxygen transport in our study. The increase in Hct may be due to the transient hemoconcentration resulting from the plasma volume shifts during exercise, a commonly observed phenomenon in acute exercise studies (29). Since greater erythrocyte deformability enhances oxygen transport to muscle capillaries even at high levels of Hct, it is possible to interpret the increase in RBC

deformability that we observed in response to NW exercise in our case as an adaptive mechanism (12).

Previous studies have shown that the effects of exercise on RBC deformability vary depending on exercise type, intensity, and subject characteristics. Moderate-intensity one-session aerobic exercise, such as NW, may enhance RBC flexibility via mechanisms involving membrane fluidity and the nitric oxide (NO) signaling pathway (30). Tomschi et al. reported no significant sex diversity in RBC deformability among elite athletes (31). In agreement with our findings, a 4-week regimen of NW exercise resulted in an enhancement of erythrocyte deformability in pre-diabetic individuals, as previously shown in our laboratory (21). However, the current research is the first to address the influence of a single session of NW exercise on blood rheology in patients with pre-hypertensive post-menopausal women. One session of NW exercise under the supervision of a physiotherapist was shown to improve the deformability values in the current study. A statistically significant change in RBC deformability was only observed at an exceptionally high shear stress, which diminishes the practical relevance of this finding. However, because this was a pilot study reporting hemorheological changes in participants, further research on this topic involving larger populations is also needed.

On the other hand, the serum levels of oxidative stress markers (TAS, TOS, and OSI) did not significantly change after a single NW session. There are a limited number of articles examining the connection between NW exercise and oxidative stress. Furthermore, the effect of long-term NW exercise was also investigated in these studies. Cebula et al. demonstrated that 6 weeks of NW exercise generated an improvement in the antioxidant defense, while the TOS levels did not significantly change (32). However, Kortas et al. demonstrated that 3 times/a week, 12 weeks of NW training caused a significant reduction in oxidative stress (9). Pilch et al. showed a significant increment in the TAS levels after 12 weeks of NW with no statistically significant changes in the TOS levels (8). Our results are particularly notable as this is the first article to examine the acute effects of NW exercise in post-menopausal patients with pre-hypertension. However, in a previous study conducted in our laboratory, it was found that a single eccentric isokinetic exercise session had no effect on oxidative stress measures (22). This is in agreement with several acute exercise studies reporting no immediate alterations in the oxidative status following moderate-intensity exercise. When evaluated in the context of existing literature, the lack of change in TAS, TOS, and OSI levels following a single session of exercise may be speculated to result from the exercise duration and intensity

not reaching a threshold sufficient to elicit a measurable impact on oxidative stress mechanisms.

Similarly, the adipokines, irisin and leptin did not show significant changes after the exercise session. Leptin is involved in appetite regulation and energy homeostasis, with levels typically elevated in obesity and reduced following sustained exercise or weight loss. Similar to our findings, obese women's leptin levels were not significantly changed by 45 minutes of walking at 60-80% HRmax (33). Additionally, leptin concentrations did not change after exercise sessions lasting 60 minutes (34). However, acute exercise interventions, particularly those of short duration or lower intensity, often fail to elicit measurable changes in leptin levels. Our findings are consistent with those of previous studies in which leptin concentrations remained stable following a single bout of aerobic exercise in women.

Irisin, a myokine linked to energy expenditure and metabolic regulation, has shown inconsistent responses to exercise. Some studies suggest increases in irisin levels following acute high-intensity or long-duration aerobic exercise, whereas others report no significant changes. A meta-analysis by Fox et al. in 2017 found that irisin levels typically rose within 15 minutes, referred to as "immediate" -after an acute exercise session (35). Similarly, Rashti et al. showed that post-menopausal women's serum irisin levels were considerably increased by vigorous concurrent circuit exercise, which was performed three times a week for 50-65 minutes per session over a period of 10 weeks (36). However, in our study, conducted at 40-55% HRmax, the exercise intensity and session may have been insufficient to stimulate significant irisin release. Furthermore, individual variability in fitness levels and muscle mass may also influence the irisin response.

CONCLUSION

In conclusion, a single session of moderate-intensity NW exercise produced significant improvements in erythrocyte deformability and hematocrit levels, suggesting beneficial acute hemorheological adaptations in post-menopausal women with pre-hypertension. However, no significant changes were observed in the oxidative stress markers, leptin, or irisin levels. The observed reduction in blood pressure may reflect post-exercise hypotension. These findings emphasize the potential of NW as a non-pharmacological strategy for vascular health promotion in this population. Our results could be used as a guide for coaches and exercise physiologists in future research using this workout regimen.

STUDY LIMITATIONS

The limitations of our study include the small sample size and the focus on the effects of a single session. The lack of post-exercise blood lipid profile assessment represents a limitation of this study. Incorporating such analyses—particularly focusing on the composition of circulating fatty acids—could have offered a more comprehensive insight into the mechanisms underlying exercise-induced changes in erythrocyte deformability. Future research should include more detailed investigations with post-exercise lipid profiling to better elucidate these mechanisms. Although a significant change was observed at a single shear stress level of RBC deformability, future studies with repeated measurements and larger sample sizes are needed to confirm this finding.



Ethics Committee Approval	The study was conducted in accordance with the latest revision of the Declaration of Helsinki and the approved Pamukkale Non-Interventional Clinical Research Ethics Committee (E-60116787-020/5756). All participants were informed about the study and tests and their written consent was obtained.
Peer Review	Externally peer-reviewed.
Authors Contributions	Conception/Design of Study- E.T, E.K.T., F.U., Y.T.Y.; Data Acquisition E.K.T., E.T.; Data Analysis/Interpretation: E.K.T., A.C.D.; Drafting Manuscript- E.K.T., Y.T.Y.; Critical Revision of Manuscript- E.K.T., M.B.K., E.T., F.U.; Final Approval and Accountability- E.K.T., M.B.K., E.T., F.U., Y.T.Y., A.C.D.
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