Efficacy of Triticum aestivum L. versus Placebo on Vascular Function in Mexican Adults with Obesity

Abel Chávez-Guerrero, MD[1]; Sara Pascoe-González, PhD[2]; María G. Ramos-Zavala, PhD[2]; Emmanuel A. Flores-Hernández, MD[1]; Miguel A. Cárdenas-Sosa, MSc[1]; Priscila D. Ramírez-Ceballos, MD[1]; Ernesto G. Cardona-Muñoz, PhD[2]; David Cardona-Müller, PhD[2]; Ana R. Rincón-Sánchez, PhD[3]; Raquel Echavarria, PhD[4]; Sandra O. Hernández-González, PhD[2].

1. Instituto Mexicano del Seguro Social, Centro Médico Nacional de Occidente, Unidad Médica de Alta Especialidad, Hospital de Especialidades, Unidad de Investigación Biomédica 02, Guadalajara, Jalisco, México.
2. Instituto de Terapéutica Experimental y Clínica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México.
3. Instituto de Biología Molecular en Medicina y Terapia Génica. Departamento de Biología Molecular y Genómica. Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México.
4. SECIHTI, Departamento de Ciencias de la Salud, Centro Universitario de los Altos, Universidad de Guadalajara, Tepatitlán de Morelos, Jalisco, México.

Short title:*Triticum aestivum* L on Vascular Function.

Correspondence: **“**Sandra Ofelia Hernández González”
E-mail: dra_sandy2003@yahoo.com.mx
Address: Belisario Domínguez #1000, Col. Independencia,
CP. 44380, Guadalajara, Jalisco, México.

Abstract

Background: Obesity is a multifactorial disease characterized by sustained inflammation that drives pathophysiological mechanisms leading to vascular dysfunction and eventual cardiovascular disease. Effective therapies to optimize vascular function are essential. Objective: To evaluate the efficacy of *Triticum aestivum* L supplementation versus placebo on vascular function in Mexican patients with obesity.

Methods: We conducted a randomized, triple-blind, placebo-controlled clinical trial in obese Mexican adults from the Guadalajara metropolitan area, diagnosed per World Health Organization criteria, who provided written informed consent. Sample size was calculated using standard clinical-trial formula, with a confidence level of 95%, statistical power of 80%, resulting in n = 10 per group. Participants were randomized to receive 500 mg *Triticum aestivum*L or matching placebo capsules every 12 hours for 120 days. Vascular function was assessed using VP1000 (ABI, baPWV) and UNEX EF (FMD), which measure blood pressure, pulse transit time, and arterial dilation after reactive hyperemia. Data are presented as mean ± SD and were analyzed using paired or independent Student's t tests, as appropriate; $p \leq 0.05$ was considered statistically significant.

Results: Twenty participants completed the study (10 per group). Mean age was higher in the *Triticum aestivum* L. group than in the placebo group (46.1 ± 4.7 vs. 36.3 ± 5.7 years; $p = 0.01$). Sixteen participants were women. After 120 days, the *Triticum aestivum* L. group showed significant reductions in waist circumference (99.1 ± 9.75 to 94.4 ± 10.85 cm; $p< 0.001$), waist-to-hip ratio (0.85 ± 0.10 to 0.82 ± 0.11; $p< 0.001$), and serum uric acid levels (5.86 ± 1.07 to 5.28

± 1.05 mg/dL; p = 0.023). An increase in flow-mediated dilation was observed, rising from 7.10 ± 2.53% at baseline to 9.15 ± 4.62% at day 120 (p = 0.06).

Conclusions: Supplementation with Triticum aestivum L. was associated with improvements in anthropometric parameters, including waist circumference and waist-to-hip ratio, as well as markers of vascular stiffness and endothelial function, and reductions in serum uric acid levels in adults with obesity. These findings suggest potential vascular benefits of Triticum aestivum L. as a nutraceutical intervention; however, larger randomized controlled trials are needed to confirm these results.

Keywords:obesity; *Triticum aestivum L.*; vascular function; flow-mediated dilation; pulse wave velocity; ankle-brachial index.

Registration: www.ClinicalTrials.gov (NCT06950138)

Introduction:

Obesity is a major global public health problem and one of the most prevalent diseases today [1]. It imposes a substantial burden on health systems by significantly increasing morbidity and mortality [2]. It also induces cardiometabolic alterations, incluidinghypertension, diabetes mellitus, dyslipidemia, and fatty liverthat collectively contribute to cardiovascular disease (CVD) and mortality [3]. CVD is the leading cause of death [4], arising from alterations in vascular structure and function [3].

Obesity is strongly associated with CVD [5]. Visceral fat hypertrophy and hyperplasia activate molecular pathways that damage the endothelium [6], promoting arterial stiffness, a progressive loss of vascular elasticity influenced by endocrine, inflammatory, immune, and extracellular matrix–related factors [7,8]. Considered an early marker of cardiovascular disease [9,10], arterial

stiffness is recognized in European hypertension guidelines as an independent risk factor that likely precedes clinical hypertension [11]. Obesity disturbs the normal balance in which nitric oxide (NO) restrains endothelin-1 (ET-1); hyperinsulinemia, RAAS activation, oxidative stress, and AGEs reduce NO, elevate ET-1, and drive vascular remodeling and arterial stiffness [7].Persistent hyperinsulinemia further suppresses endothelial NO, boosts ET-1, and promotes vasoconstriction, hypertension, and structural vessel changes [12]. Pulse wave velocity (PWV)measured by tonometry, plethysmography, or oscillometryremains the gold-standard indicator of arterial stiffness, reflecting vessel wall properties and blood viscosity [7,13]. Values >10 m/s[11]for carotid–femoral PWV or ≥12.5 m/s[14] for brachial–ankle PWV signify increased stiffness. Additional non-invasive markers include the augmentation index for wave reflection [15], flow-mediated dilation with its baseline and maximum arterial diameters for endothelial function [16,17], and the ankle-brachial index for peripheral arterial disease [18]. No targeted therapy for vascular dysfunction exists[19]; current management seeks to slow endothelial decline by addressing underlying mechanisms [20]. Pharmacologic options including ACE inhibitors, statins, and antidiabetic agents such assodium–glucose cotransporter 2 (SGLT2) inhibitors, offer antihypertensive, antioxidant, lipid-lowering, and insulin-sensitizing effects that mitigate arterial stiffness and improve endothelial function [4,20]. A healthy lifestyle, including smoking cessation, regular exercise, weight control, adequate sleep, and a balanced diet, remains the cornerstone of prevention and care [4].

Flavonoids are promising antioxidants that improve vascular function by enhancing ROS scavenging, chelating metal ions, and inhibiting lipid oxidation [21-24].*Triticum aestivum* L. (wheatgrass; TA)is a flavonoid-rich nutraceutical with a good safety profile[25,26]; nutraceuticals with similar compositions have been linked to lower arterial stiffness and

augmentation index [27-29]. Pre-clinical studies show wheatgrass reduces glucose, lipids, and weight gain [30,31], and a small randomized trial found that 814 mg day of flavonoids from dark chocolate and cocoa increased brachial arterial diameter and decreased stiffness in middle-aged women [32].No clinical studies have evaluated the effects of TA on vascular function in patients with obesity, and existing studies on other flavonoid-rich nutraceuticals in this population remain inconclusive [33].

Aim. The primary aim of this randomized, triple-blind, placebo-controlled clinical trial was to determine whether 120 days of TA supplementation improves endothelial function, assessed by brachial artery flow-mediated dilation (FMD), compared with placebo, in Mexican adults with obesity. Secondary aims were to evaluate the effects of supplementation on arterial stiffness and related vascular indices (brachial–ankle pulse wave velocity [baPWV] and ankle–brachial index [ABI]), blood pressure, anthropometric measures, and selected laboratory biomarkers.

Hypotheses. We hypothesized that, compared with placebo, TA supplementation would (I) increase FMD (improve endothelial function) and (II) attenuate arterial stiffness (lower baPWV), alongside favorable changes in central adiposity markers.

Methods:

A randomized, triple-blind (participant, investigators, and analyst), placebo-controlled clinical trial was conducted in 20 patients with obesity, defined according to WHO criteria as a body mass index (BMI) ≥30 and <40 kg/m². Participants had stable weight during the 3 months prior to enrollment and were recruited from the Guadalajara metropolitan areaand met predefined eligibility criteria.None were sedentary or engaged in extreme physical activity. All denied the use of dietary supplements or medications affecting lipid or carbohydrate metabolism. Pregnant women or those with suspected pregnancy were excluded, as were individuals with a history of

active smoking or known hypersensitivity to the Poaceae (Gramineae) family (which includes cereals such as corn, rice, barley, and oats) [34]. Additional exclusion criteria included a history of substance abuse (including alcohol), coronary, cerebrovascular, hepatic, thyroid, or renal diseases, diabetes, hypertension, dyslipidemia, infectious diseases, or the presence of pacemakers or any permanent metallic or bioelectronic devices that could interfere with bioelectrical impedance analysis.

Sample size was calculated a priori using the standard formula for randomized clinical trials as described by Jeyasselan[35], and was informed by the trial of West et al., which reported changes in brachial artery diameter parameters following cocoa/flavanol supplementation in overweight adults [32]. At the time of protocol development, robust variance estimates for flow-mediated dilation (FMD) obtained with semi-automated ultrasound systems (UNEX EF) in a comparable population were not available in our setting. Therefore, vascular ultrasound diameter measures reported by West et al. were used as the closest validated surrogate to approximate the expected intervention effect and variability. Using a two-sided alpha of 0.05 and a statistical power of 80%, the minimum required sample size was 8 participants per group. To account for an anticipated attrition rate of approximately 20%, a total of 10 participants per group were enrolled.

During the screening phase, patients were scheduled for an initial evaluation after a 12-hour overnight fast. This included a clinical history review to assess eligibility criteria and a complete physical examination. Height and weight were measured with participants wearing light clothing and no shoes. Height was determined to the nearest centimeter using a wall-mounted stadiometer (Seca 213). Weight was measured using a multi-frequency segmental body composition analyzer MC-78 TANITA (Tokyo, Japan). Body mass index (BMI) was calculated as weight in kilograms

138 divided by height in meters squared (kg/m²). Waist and hip circumferences were measured using
139 a clinical measuring tape. The waist-to-hip ratio (WHR) was calculated by dividing waist
140 circumference (WC) by hip circumference (HC), both measured in centimeters. Blood pressure
141 was assessed after a 5-minute rest period with the participant seated, using a digital
142 sphygmomanometer (OMRON HEM-9200T).
143 Venous blood samples were collected to measure serum concentrations of glucose, urea,
144 creatinine, uric acid, total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C),
145 triglycerides, very-low-density lipoprotein cholesterol (VLDL-C), alanine aminotransferase
146 (ALT), and aspartate aminotransferase (AST) using the Vitros XT7600 analyzer. Complete
147 blood counts were performed with the SYSMEX XN-2000 system using Sulfolyser, Lysercell
148 WNR, and Lysercell WDF reagents to assess safety. The atherogenic index was calculated using
149 the Castelli index (total cholesterol/HDL-C). For ET-1 and NO measurements, blood samples
150 were centrifuged, and serum was aliquoted and stored at –80°C until analysis. ET-1
151 concentrations were measured using the Human EDN1 (Endothelin-1) ELISA Kit (Enzyme-
152 Linked Immunosorbent Assay), and NO levels were quantified using the Oxford Biomedical
153 Research Colorimetric Nitric Oxide Assay Kit.
154 Seven days later, once initial laboratory results were available, inclusion criteria were confirmed,
155 anthropometric measurements were repeated, and vascular function tests were performed. These
156 included measurement of the ABI and baPWV using the VP1000 device (Omron, Japan), which
157 employs the oscillometric technique with pneumatic cuffs placed on both arms and ankles to
158 simultaneously record blood pressure and pulse transit time between the brachial and posterior
159 tibial arteries. Endothelial function was assessed using the UNEX EF device (Unex, Japan), a
160 semi-automated B-mode ultrasound system that measures baseline arterial diameter BAD and

MAD following reactive hyperemia induced by cuff release. FMD was calculated as the percentage change from BAD to MAD, reflecting endothelium-dependent vasodilation.

Participants were randomized using a sealed-envelope system into Group A or B to receive, orally, 500 mg of TA or placebo (calcined magnesia) every 12 hours. TA was obtained in lyophilized and encapsulated form and was provided free of charge by TRIGANA® (Mexico) for research purposes only. TRIGANA® had no role in the study design, data collection, data analysis, interpretation of the results, or manuscript preparation. Both interventions were lyophilized, matched in organoleptic properties, and packaged in identical plastic bottles containing 60 capsules for 30 days.

Dose rationale. We chose 500 mg twice daily (1 g per day) because a nine-month trial in children and adolescents with β-thalassemia major showed that TA capsules 250 mg three times daily (0.75 g per day) improved ferritin and red-cell indices without adverse effects, indicating long-term tolerability at a comparable dose [36]. A slightly higher total dose for a shorter period therefore offers a conservative yet pharmacologically active exposure for adults with obesity.

At the initial visit, all participants received nutritional counseling based on the "Plato del Buen Comer" (Mexican dietary guidelines), without a specific hypocaloric diet. They were also provided with a treatment and dietary adherence diary to monitor compliance over time. These tools facilitated the identification of behavioral patterns, barriers, and facilitators throughout the intervention.

The study lasted 120 days. At each 30-day visit, participants received a new bottle of capsules. Therapeutic adherence was assessed by capsule count and considered effective when more than 80% of the capsules had been consumed. Tolerability, adverse events, anthropometric and

clinical parameters, and laboratory samples were also evaluated at each visit. Vascular function tests were repeated only at the final visit on day 120.

Statistical analyses accounted for the repeated nature of the measurements (baseline and day 120) and were conducted to compare changes over time between the Triticum aestivum L. and placebo groups. Continuous variables are presented as mean ± standard deviation, and categorical variables as frequencies and percentages. Data distribution was assessed using the Shapiro–Wilk test. Within-group comparisons were performed using paired Student's t tests, and between-group comparisons were conducted using independent Student's t tests.

Secondary outcomes were analyzed using the same statistical approach. As these analyses were exploratory, results were interpreted cautiously, with greater emphasis placed on effect sizes than on statistical significance alone.

All statistical analyses were performed using IBM SPSS software (version 25 or later). A two-sided p value ≤ 0.05 was considered statistically significant.

Age eligibility criteria were predefined to minimize confounding related to vascular aging. Participants older than 60 years were excluded, as clinically relevant increases in arterial stiffness and endothelial dysfunction are more pronounced after this age. Therefore, the enrolled population represents a relatively homogeneous adult group in which age-related vascular changes are expected to be limited.

The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Council for Harmonisation (ICH) guidelines for Good Clinical Practice. It was reviewed and approved by the Local Research Ethics Committee 1301 of the Unidad Médica de Alta Especialidad, Hospital de Especialidades, Centro Médico Nacional Lic Ignacio García Téllez (approval number R-2023-1301-177) and registered at

www.ClinicalTrials.gov(NCT06950138). Written informed consent was obtained from all participants prior to enrollment.

Results

Twenty-seven individuals met the inclusion criteria and were randomized. The study flow, from the screening phase to the number of participants included, is shown in Figure 1.

A total of 20 participants were included in the final analysis: 10 in the TAintervention group and 10 in the placebo group. The mean age was 46.1 ± 4.7 years in the TAgroup and 36.3 ± 5.7 years in the placebo group ($p = 0.01$). There were 16 women in total 7 in the TA group and 9 in the placebo group.

Baseline characteristics, as well as clinical, anthropometric, laboratory, and vascular function variables, were evaluated. Patients in the TA group showed statistically significant reductions in WC, WHR, and uric acid levels compared with baseline measurements. No significant changes were observed in the remaining clinical, anthropometric, laboratory, safety, or vascular function variables (Table 1).

Efficacy in Primary Variables

FMD improved in the group receiving TA ($p = 0.06$) (Figure 2). In the placebo group, only the ABI changed significantly ($p = 0.05$) (Figure 3). No significant differences were observed between TA and placebo groups after 120 days of intervention in the following variables: systolic blood pressure (SBP) 118.3 ± 8.2 vs 122.2 ± 11.1 mmHg ($p = 0.39$); diastolic blood pressure (DBP) 68.1 ± 4.7 vs 68.7 ± 5.5 mmHg ($p = 0.79$); mean arterial pressure (MAP) 84.8 ± 5.6 vs 86.5 ± 2.27 mmHg ($p = 0.57$); baPWV 12.28 ± 1.73 vs 13.74 ± 1.62 m/s ($p = 0.68$); MAD 3.74 ± 0.47 vs 3.88 ± 0.74 mm ($p = 0.62$); and BAD 3.39 ± 0.36 vs 3.62 ± 0.72 mm ($p = 0.37$). In the between-group analysis, participants receiving TA had a significant reduction

in WHR ($p = 0.042$), whereas in the placebo group, brachial–ankle pulse wave velocity values were higher at day 120 compared with baseline ($p = 0.06$).

No abnormalities were observed in hepatic or renal markers. Creatinine and liver enzyme levels remained within normal ranges throughout the study. No adverse events were reported, and 100% of randomized participants demonstrated good therapeutic adherence. The adherence diary revealed a positive effect on treatment compliance suggesting an increase in patient awareness and responsibility, reflected in improved dietary quality. Physical activity was assessed by self-report and showed no relevant changes. Seven participants withdrew from the study, four from the TA group and three from the placebo group, all due to voluntary withdrawal.

Discussion

This is the first randomized, triple-blind, placebo-controlled clinical trial to evaluate the effects of TA supplementation on vascular function parameters in Mexican adults with obesity. In addition, the present study employed the semi-automated UNEX EF device to assess flow-mediated dilation (FMD), a method with validated reproducibility [37]. Our findings indicate that 120 days of supplementation with TA were associated with significant reductions in waist circumference and waist-to-hip ratio. Furthermore, the increase observed in FMD, together with the attenuation of brachial–ankle pulse wave velocity compared with the placebo group, suggests a potential favorable effect on endothelial function.

Previous studies have described the antioxidant and anti-inflammatory properties of TA, largely attributed to its high polyphenol and flavonoid content, which may modulate vascular responses to oxidative stress, a key contributor to endothelial dysfunction associated with obesity [38]. Consistent with these findings, other flavonoid-rich nutraceuticals, such as quercetin and

resveratrol, have been reported to improve arterial stiffness and endothelial function in both human and animal models [39,40].

Although no statistically significant changes were detected in circulating nitric oxide (NO) or endothelin-1 (ET-1) concentrations after 120 days of intervention, the maintenance of NO levels and the absence of deterioration in ET-1, particularly in the TA group, may be consistent with the stabilization of endothelial function observed in the functional vascular assessments. Circulating concentrations of these biomarkers are influenced by multiple systemic factors and may not fully reflect local endothelial activity. Consequently, functional vascular measures such as FMD may represent more sensitive and earlier indicators of endothelial modulation, particularly in pilot or short-term interventions, than systemic NO or ET-1 levels alone [41]. The modest increase in NO, together with the attenuation of ET-1 elevation, suggests that TA supplementation may contribute to a more favorable balance of vasoactive mediators, potentially enhancing endothelial function and reducing vascular tone. This interpretation is consistent with the observed increase in FMD and stabilization of baPWV, despite the natural tendency for arterial stiffness to progress in individuals with obesity. These findings support the hypothesis that polyphenolic compounds present in Triticum aestivum L. may exert protective vascular effects through modulation of endothelium-derived factors [42]

The observed changes in vascular function parameters, including PWV and FMD, may be partially explained by reductions in abdominal adiposity, as evidenced by decreased waist circumference and improvement in waist-to-hip ratio, along with a slight reduction in body weight [43]. Central adiposity is closely linked to endothelial dysfunction and arterial stiffness, and even modest reductions may translate into measurable vascular benefits.

Importantly, no clinically relevant adverse effects or abnormalities in liver or renal function were observed, which is consistent with previous studies demonstrating the good tolerability of TA as a dietary supplement [44]. The excellent therapeutic adherence observed further supports favorable patient acceptability and reinforces the safety profile of this intervention.

The reduction in serum uric acid represents another relevant finding, given the association between hyperuricemia, endothelial dysfunction, and increased cardiovascular risk in individuals with obesity [45]. This effect may be mediated by the ability of TA to reduce oxidative stress and inflammation, mechanisms previously described for other antioxidant compounds [46].

Several limitations of this study should be acknowledged. Although the sample size was calculated a priori using a standard clinical trial formula and was considered adequate to detect clinically meaningful changes in endothelial function, the overall number of participants was relatively small. The sample size estimation was informed by arterial diameter parameters reported in previous studies; however, FMD was predefined as the primary clinical endpoint because it reflects endothelium-dependent vasodilation and is a validated surrogate marker of vascular health. Larger studies will be required to confirm these findings and to refine power calculations using FMD-specific variance estimates derived from the present study.

In addition, the duration of the intervention may have limited the detection of structural vascular changes. While improvements in functional vascular parameters have been reported within weeks using other flavonoid-rich nutraceuticals, reductions in arterial stiffness often require longer follow-up periods, as observed in studies involving antihypertensive therapies [47]. Similarly, short-term supplementation with cocoa-derived flavanols has not consistently demonstrated changes in PWV or related vascular markers, underscoring the importance of intervention duration [33].

Although the randomized and blinded design strengthens internal validity, the findings should be interpreted with caution and may not be generalizable to other populations. The use of self-reported dietary intake and physical activity data may also have introduced information bias. Despite the observed difference in mean age between groups, all participants were younger than 60 years, an age range in which major structural vascular changes and pronounced increases in arterial stiffness are less prominent, reducing the likelihood of age as a major confounder. The predominance of women reflects the typical profile of participants in obesity-related preventive studies, and available evidence suggests that sex-related differences in arterial stiffness and endothelial function are modest in middle-aged adults without overt cardiovascular disease. Nevertheless, future studies with larger and more balanced cohorts are warranted to further explore the potential influence of age and sex.

Conclusion

Supplementation with TA for 120 days in Mexican adults with obesity was associated with significant improvements in WC, WHR, and serum uric acid levels. The increase observed in FMD, together with the attenuation of PWV progression compared with the placebo group, suggests a potential favorable effect on endothelial function. These preliminary findings support the potential role of TA as a nutraceutical intervention in the prevention of obesity-related cardiovascular complications.

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Conflict of interest

The authors declare no conflict of interest.

References

1. World Health Organization. Cardiovascular diseases. Published 2022. Accessed July 14, 2025. https://www.who.int/health-topics/cardiovascular-diseases
2. World Heart Federation. What is cardiovascular disease? Published 2022. Accessed July 14, 2025. https://world-heart-federation.org/what-is-cvd/
3. Safaei M, Sundararajan EA, Driss M, Boulila W, Shapi'i A. A systematic literature review on obesity: understanding the causes and consequences of obesity and reviewing various machine-learning approaches used to predict obesity. Comput Biol Med. 2021;136:104754. doi:10.1016/j.compbiomed.2021.104754
4. Visseren F, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, Capodanno D, Cosyns B, Crawford C, Davos CH, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021;42:3227–3337. doi:10.1093/eurheartj/ehab484
5. Haidar A, Horwich T. Obesity, cardiorespiratory fitness, and cardiovascular disease. Curr Cardiol Rep. 2023;25:1565–1571. doi:10.1007/s11886-023-01975-7
6. Lin X, Li H. Obesity: epidemiology, pathophysiology, and therapeutics. Front Endocrinol (Lausanne). 2021;12:706978. doi:10.3389/fendo.2021.706978
7. Para I, Albu A, Porojan MD. Adipokines and arterial stiffness in obesity. Medicina (Kaunas). 2021;57:653. doi:10.3390/medicina57070653
8. Kim HL. Arterial stiffness and hypertension. Clin Hypertens. 2023;29:31. doi:10.1186/s40885-023-00258-1
9. Koenen M, Hill MA, Cohen P, Sowers JR. Obesity, adipose tissue and vascular dysfunction. Circ Res. 2021;128:951–968. doi:10.1161/CIRCRESAHA.121.318093

10. Miyoshi T, Ito H. Arterial stiffness in health and disease: the role of cardio-ankle vascular index. J Cardiol. 2021;78:493–501. doi:10.1016/j.jjcc.2021.07.011
11. Reference Values for Arterial Stiffness' Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: establishing normal and reference values. Eur Heart J. 2010;31:2338–2350. doi:10.1093/eurheartj/ehq165
12. Kosmas CE, Bousvarou MD, Kostara CE, Papakonstantinou EJ, Salamou E, Guzman E. Insulin resistance and cardiovascular disease. J Int Med Res. 2023;51:3000605231164548. doi:10.1177/03000605231164548
13. Pilz N, Heinz V, Ax T, Fesseler L, Patzak A, Bothe TL. Pulse wave velocity: methodology, clinical applications, and interplay with heart-rate variability. Rev Cardiovasc Med. 2024;25:266. doi:10.31083/j.rcm2507266
14. Lu Y, Kiechl SJ, Wang J, Xu Q, Kiechl S, Pechlaner R, Chen Z, Xu X, Paudel D, Di Angelantonio E, et al. Global distributions of age- and sex-related arterial stiffness: systematic review and meta-analysis of 167 studies with 509 743 participants. EBioMedicine. 2023;92:104619. doi:10.1016/j.ebiom.2023.104619
15. Russell SL, Rahman M, Steward CJ, Harwood AE, McGregor G, Banerjee P, Okwose N, Jakovljevic DG. Central pulse wave velocity and augmentation index are repeatable and reproducible measures of arterial function. Health Sci Rep. 2024;7:e70155. doi:10.1002/hsr2.70155
16. Ahn Y, Aung N, Ahn HS. A comprehensive review of clinical studies applying flow-mediated dilation. Diagnostics (Basel). 2024;14:2499. doi:10.3390/diagnostics14222499

17. Holder SM, Bruno RM, Shkredova DA, Dawson EA, Jones H, Hopkins ND, Hopman MTE, Bailey TG, Coombes JS, Askew CD, et al. Reference intervals for brachial artery flow-mediated dilation and relation with cardiovascular risk factors. Hypertension. 2021;77:1469–1480. doi:10.1161/HYPERTENSIONAHA.120.15754
18. Poredos P, Stanek A, Catalano M, Boc V. Ankle–brachial index: diagnostic tool of peripheral arterial disease and predictor of cardiovascular risk—an update of current knowledge. Angiology. 2024;Epub ahead of print. doi:10.1177/00033197241226512
19. Powell-Wiley TM, Poirier P, Burke LE, Després JP, Gordon-Larsen P, Lavie CJ, Lear SA, Ndumele CE, Neeland IJ, Sanders P, St-Onge MP, et al. Obesity and cardiovascular disease: a scientific statement from the American Heart Association. Circulation. 2021;143:e984–e1010. doi:10.1161/CIR.0000000000000973
20. Engin A. Endothelial dysfunction in obesity and therapeutic targets. Adv Exp Med Biol. 2024;1460:489–538. doi:10.1007/978-3-031-63657-8_17
21. Micek A, Godos J, Del Rio D, Galvano F, Grosso G. Dietary flavonoids and cardiovascular disease: a comprehensive dose-response meta-analysis. Mol Nutr Food Res. 2021;65:e2001019. doi:10.1002/mnfr.202001019
22. Jomova K, Alomar SY, Valko R, Liska J, Nepovimova E, Kuca K, Valko M. Flavonoids and their role in oxidative stress, inflammation, and human diseases. Chem Biol Interact. 2025;413:111489. doi:10.1016/j.cbi.2025.111489
23. Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. Plant flavonoids: classification, distribution, biosynthesis and antioxidant activity. Food Chem. 2022;383:132531. doi:10.1016/j.foodchem.2022.132531

24. Fardoun MM, Maaliki D, Halabi N, Iratni R, Bitto A, Baydoun E, Eid AH, Zurayk D, Nasser SA, Abd-El-Khalick H, et al. Flavonoids in adipose-tissue inflammation and atherosclerosis: one arrow, two targets. Clin Sci (Lond). 2020;1[34]:1403–1432. doi:10.1042/CS20200356

25. Moshawih S, Abdullah Juperi RNA, Paneerselvam GS, Ming LC, Liew KB, Goh BH, Al-Worafi YM, Choo CYL, Thuraisingam S, Goh HP, et al. General health benefits and pharmacological activities of Triticum aestivum L. Molecules. 2022;27:1948. doi:10.3390/molecules27061948

26. Kaviya M, Balamuralikrishnan B, Vijaya Anand A, Sangeetha T, Senthilkumar N, Malaisamy A, Sivasamy M, Poorni L, Arun M, Abirami S, et al. Evaluation of phytoconstituents of Triticum aestivum grass extracts on nutritional attributes, antioxidant and antimicrobial activities against food pathogens with molecular in silico investigation. Food Front. 2023;4:831–848. doi:10.1002/fft2.233

27. Ciumărnean L, Milaciu MV, Runcan O, Vesa SC, Răchișan AL, Negrean V, Perné MG, Donca VI, Alexescu TG, Para I, et al. The effects of flavonoids in cardiovascular diseases. Molecules. 2020;25:4320. doi:10.3390/molecules25184320

28. Yamagata K, Yamori Y. Inhibition of endothelial dysfunction by dietary flavonoids and preventive effects against cardiovascular disease. J Cardiovasc Pharmacol. 2020;75:1–9. doi:10.1097/FJC.0000000000000757

29. Dong Y, Wu X, Han L, Bian J, He C, El-Omar E, Gong L, Wang M. The potential roles of dietary anthocyanins in inhibiting vascular endothelial-cell senescence and preventing cardiovascular diseases. Nutrients. 2022;14:2836. doi:10.3390/nu14142836

30. Im JY, Ki HH, Xin M, Kwon SU, Kim YH, Kim DK, Hong SP, Jin JS, Lee YM. Anti-obesity effect of Triticum aestivum sprout extract in high-fat diet-induced obese mice. BiosciBiotechnolBiochem. 2015;79:1133–1140. doi:10.1080/09168451.2015.1006567
31. Bueno P, Barbalho SM, Guiguer ÉL, Souza M, Medeiros I, Zattiti IV, Bueno M, Nutels GS, Goulart RA, Araújo AC. Effects of green wheat (Triticum turgidum) and common wheat (Triticum aestivum) on the metabolic profile of Wistar rats. J Med Food. 2019;22:1222–1225. doi:10.1089/jmf.2019.0089
32. West SG, McIntyre MD, Piotrowski MJ, Poupin N, Miller DL, Preston AG, Wagner P, Groves LF, Skulas-Ray AC. Effects of dark chocolate and cocoa consumption on endothelial function and arterial stiffness in overweight adults. Br J Nutr. 2014;111:653–661. doi:10.1017/S0007114513002912
33. Weigant J, Afchar A, Barzen M, Dicks L, Zimmermann BF, Schmid M, Weinhold L, Stoffel-Wagner B, Ellinger J, Stehle P, et al. Cardiometabolic impact of encapsulated cocoa powder and pure cocoa-ingredients supplementation: a comparative placebo-controlled randomized clinical trial in adults. Mol Nutr Food Res. 2025;69:e202400490. doi:10.1002/mnfr.202400490
34. Akhondi H, Ross AB. Gluten-associated medical problems. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2022.https://www.ncbi.nlm.nih.gov/books/NBK538505/
35. Jeyasselan L, Rao PSS. Methods of determining sample size in clinical trials. Indian Pediatr. 1989;26:115–121.https://pubmed.ncbi.nlm.nih.gov/2753525/

36. Desai TR, Solanki JK, Buch P, Goyal RK. Triticum aestivum (wheat-grass) formulation: an alternate treatment for patients with thalassemia. Orient Pharm Exp Med. 2008;7:466–476. doi:10.3742/OPEM.2008.7.5.466
37. Dobbie LJ, Mackin ST, Hogarth K, Lonergan F, Kannenkeril D, Brooksbank K, Delles C. Validation of semi-automated flow-mediated dilation measurement in healthy volunteers. Blood Press Monit. 2020;25:216–223. doi:10.1097/MBP.0000000000000448
38. Sharma P, Gujral N, Singh M. Wheatgrass: a functional food with therapeutic potential. J Food Sci Technol. 2020;57:1271–1279. doi:10.1007/s13197-019-04171-7
39. Arabi SM, ShahrakiJazinaki M, Chambari M, Bahrami LS, Maleki M, Sukhorukov VN, Sahebkar A. The effects of quercetin supplementation on cardiometabolic outcomes: an umbrella review of meta-analyses of randomized controlled trials. Phytother Res. 2023;37:5080–5091. doi:10.1002/ptr.7971
40. Gimblet CJ, Kruse NT, Geasland K, Michelson J, Sun M, Mandukhail SR, Wendt LH, Eyck PT, Pierce GL, Jalal DI. Effect of resveratrol on endothelial function in patients with CKD and diabetes: a randomized controlled trial. Clin J Am Soc Nephrol. 2024;19:161–168. doi:10.2215/CJN.0000000000000337
41. Stromsnes K, Mas-Bargues C, Gambini J, Gimeno-Mallench L. Protective effects of polyphenols present in Mediterranean diet on endothelial dysfunction. Oxid Med Cell Longev. 2020;2020:2097096. doi:10.1155/2020/2097096
42. Weil BR, Westby CM, Van Guilder GP, Greiner JJ, Stauffer BL, DeSouza CA. Enhanced endothelin-1 system activity with overweight and obesity. Am J Physiol Heart Circ Physiol. 2011;301:H689–H695. doi:10.1152/ajpheart.00206.2011

43. Cui C, Wu Z, Zheng J, Chu J, Zhu J, Qi Y, Zhang Z, Wang Z, Zhang M, Mo Z. Arterial stiffness and obesity as predictors of diabetes: longitudinal cohort study. JMIR Public Health Surveill. 2024;10:e46088. doi:10.2196/46088
44. Bascuñán KA, Varela-López A, Romero-Márquez JM, Citores MJ, Sáinz J, Sánchez-González C. Potential tolerability of ancient grains in non-celiac wheat-sensitivity patients: a preliminary evaluation. Front Med (Lausanne). 2022;9:995019. doi:10.3389/fmed.2022.995019
45. Saito Y, Tanaka A, Node K, Kobayashi Y. Uric acid and cardiovascular disease: a clinical review. J Cardiol. 2021;78:51–57. doi:10.1016/j.jjcc.2020.12.013
46. Petreski T, Ekart R, Hojs R, Bevc S. Hyperuricemia, the heart, and the kidneys— to treat or not to treat? Ren Fail. 2020;42:978–986. doi:10.1080/0886022X.2020.1822185
47. Wu S, Tian X, Chen S, Zhang Y, Zhang X, Xu Q, Wang A. Arterial stiffness and blood pressure in treated hypertension: a longitudinal study. J Hypertens. 2023;41:768–774. doi:10.1097/HJH.0000000000003400

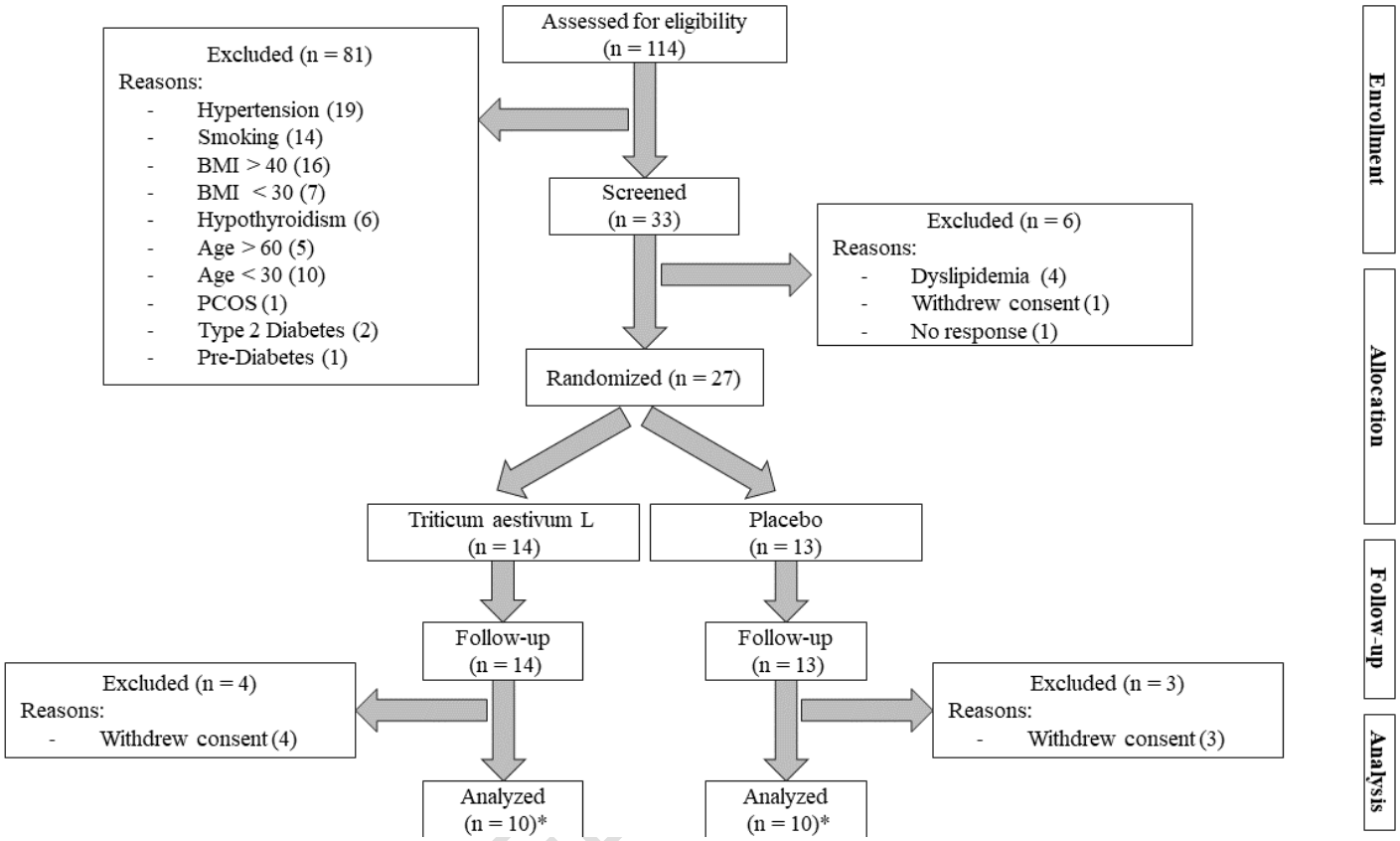

Figure 1. Flow diagram. The enrollment of 10 participants per group satisfied the minimum sample-size requirement calculated a priori to detect a clinically meaningful difference. Adapted from the CONSORT 2010 statement.

Variable	TA Baseline	TA Day 120	p	Placebo Baseline	Placebo Day 120	p
Weight (kg)	87.00 ± 13.39	85.57 ± 13.24	0.26	83.15 ± 12.60	84.28 ± 12.44	0.14
BMI (kg/m^2)	32.67 ± 2.64	32.17 ± 3.00	0.28	32.90 ± 3.12	33.35 ± 3.05	0.13
WC (cm)	99.10 ± 9.75	94.40 ± 10.85	0.00†	99.00 ± 9.38	99.20 ± 7.48	0.92
WHR	0.85 ± 0.10	0.82 ± 0.11	0.00†	0.85 ± 0.07	0.87 ± 0.09	0.34
Adiposity (%)	37.31 ± 5.61	36.64 ± 4.22	0.29	39.20 ± 3.52	38.00 ± 5.08	0.20
Uric acid (mg/dL)	5.86 ± 1.07	5.28 ± 1.05	0.02*	4.78 ± 1.12	4.75 ± 0.83	0.90
Total cholesterol (mg/dL)	180.10 ± 21.22	172.40 ± 26.68	0.19	184.20 ± 16.41	195.10 ± 32.35	0.24
LDL-C (mg/dL)	109.38 ± 24.90	103.98 ± 21.76	0.42	124.64 ± 24.48	118.14 ± 26.44	0.13
HDL-C (mg/dL)	45.80 ± 11.24	46.80 ± 10.27	0.65	52.40 ± 14.88	51.20 ± 11.68	0.74
Triglycerides (mg/dL)	114.80 ± 36.56	110.40 ± 69.23	0.80	119.70 ± 31.26	129.00 ± 39.90	0.47
Atherogenic index	4.13 ± 1.20	3.90 ± 1.15	0.18	4.33 ± 1.38	3.94 ± 0.91	0.27
Glucose (mg/dL)	87.70 ± 7.18	88.80 ± 5.49	0.56	91.70 ± 9.87	89.70 ± 12.86	0.35
Urea (mg/dL)	25.50 ± 8.30	31.30 ± 10.69	0.16	33.30 ± 9.98	32.40 ± 10.29	0.78
Creatinine (mg/dL)	0.71 ± 0.15	0.70 ± 0.13	0.81	0.71 ± 0.26	0.72 ± 0.15	0.85

AST (U/L)	27.80	±	6.82	23.40	±	7.12	0.24	29.10	±	9.71	27.30	±	7.80	0.64
ALT (U/L)	31.20	±	12.72	22.70	±	12.45	0.19	30.30	±	18.22	32.40	±	14.50	0.69
SBP (mmHg)	111.80	±	8.69	118.30	±	8.27	0.07	116.50	±	7.30	122.20	±	11.14	0.09
DBP (mmHg)	64.10	±	6.33	68.10	±	4.72	0.14	66.70	±	7.91	68.70	±	5.53	0.39
MAP (mmHg)	80.00	±	6.79	84.83	±	5.60	0.10	83.29	±	7.50	86.53	±	7.27	0.20
baPWV (m/s)	11.86	±	1.26	12.28	±	1.73	0.28	12.85	±	1.10	13.74	±	1.62	0.06
FMD (%)	7.10	±	2.53	9.15	±	4.62	0.06	4.36	±	2.22	7.46	±	3.54	0.07
BAD (mm)	3.39	±	0.62	3.38	±	0.35	0.99	3.76	±	0.56	3.62	±	0.72	0.47
MAD (mm)	3.66	±	0.59	3.74	±	0.46	0.72	3.92	±	0.54	3.88	±	0.73	0.83
ABI	1.01	±	0.08	0.99	±	0.05	0.52	1.04	±	0.05	0.98	±	0.10	0.05*
ET-1 (pg/ml)	23.19	±	4.76	23.99	±	5.96	0.67	22.78	±	7.25	24.86	±	5.32	0.35
NO (μmol/mL)	46.78	±	24.42	50.82	±	10.64	0.98	52.29	±	14.46	55.40	±	13.79	0.71

Table 1. Within-group changes after 120 days of intervention

Values are mean ± standard deviation. *p < 0.05, †p < 0.01 vs baseline (paired Student t test).

Abbreviations: ABI, ankle-brachial index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAD, basal arterial diameter; baPWV, brachial–ankle pulse wave velocity; BMI, body mass index; DBP, diastolic blood pressure; ET-1, endothelin-1; FMD, flow-mediated dilation; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MAD, maximum arterial diameter; MAP, mean arterial pressure; NO, nitric oxide;

SBP, systolic blood pressure; WC, waist circumference; WHR, waist-to-hip ratio. ET-1 and NO, reference range based on the assay standard curve (method-dependent).

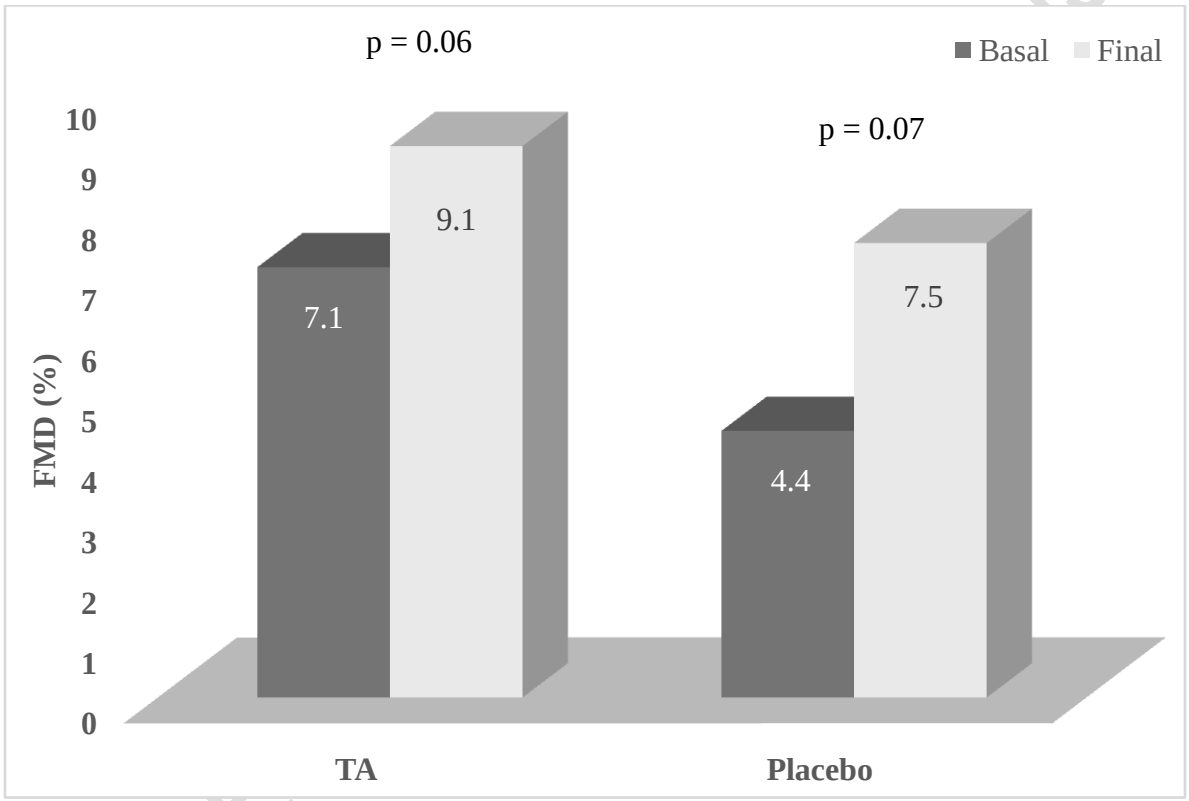

Figure 2. Basal and final flow-mediated dilatation (FMD). Change in brachial artery FMD after 120 days of supplementation. Bars show mean at baseline and day 120 for the TA and placebo groups (n = 10 per group). Within-group comparison by paired Student's t test; improvement in FMD in the TA group (p= 0.06).

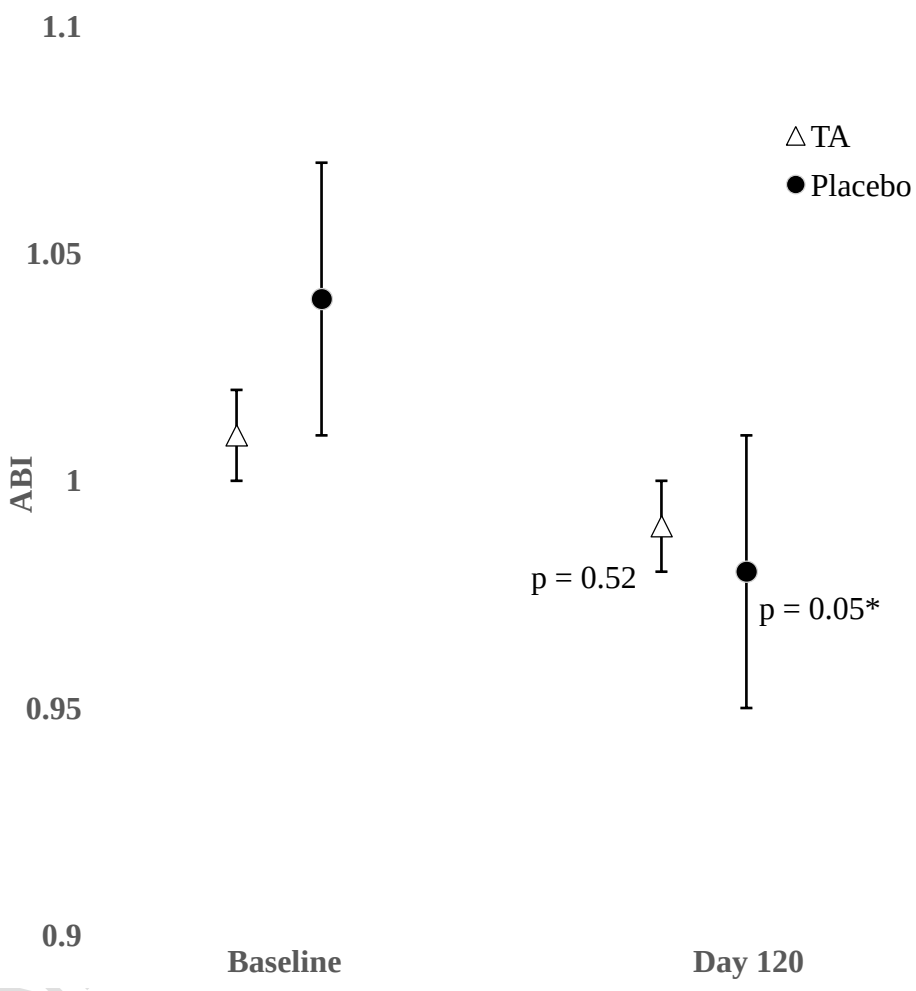

Figure 3. Ankle–brachial index (ABI) at baseline and day 120 in theTA and placebo groups. Symbols denote mean; error bars represent standard deviation. Paired Student t tests vs baseline: placebo showed a significant decrease (p = 0.05), whereas TA was not significant (p = 0.52).