

# The Metabolic Effects of Hawthorn Vinegar in Patients with High Cardiovascular Risk Group

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**Abstract** Hawthorn (*Crataegus oxycantha*) vinegar produced traditionally only in Bolu (Turkey) in small amount is believed to cure some diseases such as cold, flu and cardiovascular diseases by local people. However, very limited amount of information is available regarding the health effect of hawthorn vinegar. In order to investigate the metabolic effects of hawthorn vinegar 37 patients who were diabetic, hypertensive, overweight and taking related medications with high cardiovascular risk group were selected and without changing their diet habits and exercise program, vinegar consumption was added to each meal during four-week. There was a significant change on the weight loss, BMI change, blood pressure decrease, glucose, cholesterol, triglyceride, LDL, HDL and cholesterol/HDL after consumption of Hawthorn vinegar ( $p \leq 0.05$ ). Total phenolic substance concentration (mg/mL) and total monomeric anthocyanin content (mg/L) and total antioxidant capacity (%) of the Hawthorn vinegar were measured as 0.51, 0.25 and 77, respectively. This study demonstrates that Hawthorn vinegar has positive metabolic effects on the patients with high cardiovascular risk factors.

**Keywords:** hawthorn vinegar, metabolism, weight loss, preventive medicine

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## 1. Introduction

Hawthorn fruit has been documented as a food in China as early as the seventh millennium before Christ [1]. During the last decades, hawthorn has received much attention because of its potential to reduce plasma cholesterol and triacylglycerol (TAG) concentrations and to treat heart arrhythmia [2,3,4]. It has also been shown that hawthorn consumption has favorable effects in the early stages of congestive heart failure [3,4] angina pectoris [5,6] and reducing blood pressure [7]. In addition, hawthorn fruit significantly inhibited thromboxane A2 biosynthesis and platelet adhesion, thus reducing the formation of atheroma and thrombosis [8].

Vinegar has been used as both food and medicine in many cultures for thousands of years. Both animals and human studies indicated that vinegar has a potential antiglycemic effects. It has been shown that regular vinegar ingestion favorably influences hemoglobin A1c values in individuals with type 2 diabetes [9]. In human, the area under the insulin response curve after ingestion of sucrose was reduced by 20 % when coadministered with vinegar, and 20 mL vinegar consumption reduced the glycemic response in a mixed meal by more than 30 % [10].

The hawthorn fruit is commonly consumed as fresh fruit in Turkey, and small amount of Hawthorn vinegar

production is practiced only in Bolu Province (Turkey). In addition to regular usage, hawthorn vinegar is also consumed as hot drink by mixing warm water. In order to produce vinegar, red and yellow hawthorn fruits are used in equal quantities and fermented in oxygenated conditions for two-years in the barrels made from oak tree.

It has been accepted that atherosclerosis can be preventable and treatable disease with different drugs. Because of the duration of the therapy and major side effects due to chemical drugs in treatment of atherosclerosis; herbal medication may be suitable to substitute for these drugs. Cheap, effective and native solutions in metabolic problems are very important and strongly needed in preventive medicine especially in poor countries, and thus, alternative treatments to solve the problem are in search. Therefore, determination of the metabolic effects of hawthorn vinegar intake on biochemical and clinical risk factors of atherosclerosis in high cardiovascular risk group was prompted in this study. It was speculated that the combined metabolic effects of vinegar and hawthorn in vinegar may show an alternative way to combat with metabolic disorders and their consequences. In addition, some physiochemical properties in addition to antimicrobial effects of Hawthorn vinegar were also measured to obtain physical and chemical profile of hawthorn vinegar.

## 2. Materials and Methods

## 2.1. Hawthorn Vinegar

Vinegar samples were produced in the local producer, Kemal Unlu Sirkeleri, Bolu (Turkey). After collection, the fruits were pressed with metal press and placed in oak barrels. Pressed fruits were mixed with water until the water level covered the fruits, and fermentation was started with natural microflora. The mixture was fermented for two years for vinegar production. The mixture was filtered to separate the fruits from the liquid part at the end of the first year, and liquid part was transferred to oak barrel again to complete the fermentation for a year. After the second year, acidity of the vinegar was controlled and the fermentation was terminated. It is classified as cloudy vinegar, thus, the product was bottled without filtration.

## 2.2. Microbial Cultures

*Escherichia coli* O157: H7 (EDL 931 04054) and *Listeria monocytogenes* (NCTC 2167) cultures were obtained from Refik Saydam Hifzissihha Research Center Culture Collection Laboratory (Ankara, Turkey) in lyophilized form. Cultures of *E. coli* O157:H7 and *L. monocytogenes* were activated with three consecutive transfers into TSB (Fluka, Germany) and following incubation at 35±2°C. After activation, the culture was inoculated into vinegar samples. Culture of *Candida albicans* was obtained from Ankara University Food Engineering Department (Ankara, Turkey). The culture was activated with transferring the culture into nutrient broth as enrichment media and the culture was incubated at 22±2° for 3-5 days prior to inoculation into vinegar samples.

## 2.3. pH

pH measurement was conducted by 10 mL of the triplicate samples at room temperature by Orion perPect logR meter (Inolab WTW, Germany).

## 2.4. Titratable Acidity

Titrate acidity (% TA) measurement was performed with 10 mL of the samples titrated with 0.1N NaOH after the addition of 0.5 mL phenolphthalein until the color of the samples changed. Consumed NaOH in mL was recorded, and results were calculated with reference to acetic acid.

## 2.5. Total Soluble Solids

°Brix (total soluble solids) of the samples at room temperature was measured by 507-1 model hand-held refractometer (Nippon Optical Works Co. Ltd, Japan).

## 2.6. Conductivity

Conductivity of the samples was measured by hand held conductivity meter (Sension 5 model, HACH, CO, USA), and the results were given as mS/cm.

## 2.7. Color Measurement

Color measurement was performed by Hunter Color Flex spectrophotometer (Hunter Associates Laboratory

Inc., Reston VA, USA) by using CIELAB color scale at D65/10° to measure L\*, a\* and b\* parameters.

## 2.8. Total Monomeric Anthocyanin Content

In order to measure the total monomeric anthocyanin content, vinegar samples were diluted with 0.025 M KCl (Sigma Chemical Co., Stockholm, Sweden) and 0.04 M Na-acetate (Sigma Chemical Co., Stockholm, Sweden), separately and the mixtures were centrifuged at 2400 rpm for 2 min by vortex. The samples were set for 20 min, and both dilutions were read at both 520 and 700 nm using a spectrophotometer (Perkin Elmer Lambda 25 model, MA, USA). Results were calculated as cyanidin 3-glucoside equivalent in mg/L [11].

## 2.9. Total Antioxidant Capacity

For the total antioxidant capacity (%) analysis, Tris-HCl tampon at pH 7.4 was added to 0.1 mL of the samples, mixed at 2400 rpm for 5 min by vortexing and then 1mL of DPPH (prepared in ethanol) was added. The absorbance of vinegar samples was measured at 517 nm wavelength after 30 min [12].

## 2.10. Total Phenolic Content and Identification of Phenolic Compounds

Total phenolic content of the samples was measured by the spectrophotometric method [13]. Diluted samples were filtered through 0.45 µm filter, 5 mL of 0.2 N Folin-Ciocalteu was added to 1 mL of the filtered samples, and then mixed by vortexing. After that, a 4 mL of saturated sodium carbonate was added and the mixture was placed in a water bath adjusted to 50±5°C for 5 min. Absorbance of the samples were measured at 760 nm after quick cooling down the sample temperature to room temperature. Obtained absorbance values were calculated from the gallic acid standard curve prepared with 100, 200, 300, 400 and 500 mg/L gallic acid. The total phenolic content of the samples were calculated as mg/L gallic acid equivalence.

Identification and quantification of phenolic compounds was performed by a high-performance liquid chromatography (Shimadzu, Kyoto, Japan) equipped with a LC-10ADvp pump, an SIL-10ADvp auto sampler, a DAD detector, an SCL-10Avp system controller, a DGU-14A degasser, a CTO-10Avp column oven, and a normal phase silica gel column and a reversed-phase (C18) silica gel column (Radial-pak cartridge, 10 cmx 8 mm I.D., 4 µm particle size, Waters, Milford, MA, USA). Linear gradient solvent system consisted of solvents A and B (5% and 25% acetonitrile in 25 mM sodium phosphate buffer at pH 2.4) with a flow rate of 1 mL/min was used. Solvent B proportion was increased from 10% to 80% during the first 20 min, held for 10 min, and then returned to 10% in the last 5 min. Monitoring of the effluent was performed at 278 nm for procyanidin Bz (PC-Bz) and (-)-epicatechin (EC), and 360 nm for chlorogenic acid (ChA), hyperoside (HP) and isoquercitrin (IQ). Calibration curves prepared from the standards were used for the quantification of phenolic compounds. These results presented >0.990 correlation with calibration curves [14].

## 2.11. Metal Ion Concentration

Metal ion concentration (mg/L) of hawthorn vinegar samples were measured by atomic absorption spectrophotometer (Perkin Elmer A Analyst 400 Atomic Absorption Spectrophotometer, USA). Two mL of the samples were mixed with 65% HNO<sub>3</sub> and 2 mL H<sub>2</sub>O<sub>2</sub> and placed in microwave oven (Berghof Speedwave MWS-2, Germany) to burn the samples by 3 step procedure. The samples were started to be burned at 145°C for 5 min with 75% power then temperature increased to 180°C for 10 min with 90 % power and finally at 100°C for 10 min for 40% power.

## 2.12. Antimicrobial Activity

Antimicrobial activity of hawthorn vinegar samples was tested against *E. coli* O157:H7, *L. monocytogenes* and *C.albicans*. After activation of the cultures were completed, they were transferred into separate test tubes containing 0.1% peptone water in order to have 10<sup>6</sup>-10<sup>7</sup> cfu/mL initial cell number. Five, 20, 40, 80, 160 and 320 µL/mL of hawthorn vinegar were added to test tubes, and the tubes were mixed by vortexing. Samples were diluted with 0.1% peptone water, appropriate dilutions for *E. coli* O157:H7 were plated on MacConkey Sorbitol Agar (MSA) by surface plating method, and incubated at 35±2°C for 24 h. Appropriate dilutions for *L. monocytogenes* were plated onto Oxford Listeria Selective (Basis) Agar (OA), and the plates were incubated at 35±2°C for 24 h. Dilutions for *C. albicans* were plated on Yeast Extract Agar (YEA) and the plates were incubated at 22±2° for 3-5 days. Results were given as log cfu/mL.

## 2.13. Metabolic Effect of Hawthorn Vinegar

Thirty seven volunteers (21 female and 16 males) were selected for the study. All patients were screened through medical history and physical examination in Abant Izzet Baysal University Faculty of Medicine (Bolu, Turkey). All patients were Tip 2 diabetic and taking regularly oral antidiabetic medicine. Twenty patients were taking oral metformine and 10 of them were also taking gliclazide. Seventeen patients were regulating blood glucose only by diet. All patients were taking Angiotensine Converting Enzyme (ACE) inhibitors or Angiotensine Receptor Blockers (ARBs) in addition to 300 mg aspirin. No change was made in their physical activities medications and diet habits during the study. Current pregnancy, breastfeeding, unwillingness to take hawthorn vinegar consumption, taking other medicine or vitamin supplements, alcohol use, infection clinic or planning holiday were taken as the exclusion criteria from the study. Decompensated heart failure, kidney or liver failure, pancreatic disease or malignancies were the other criteria for exclusion. Subjects were informed about the aim and nature of the study before giving written informed consent.

Volunteers asked to consume 20 mL of hawthorn vinegar diluted with 40 mL water just after the meals for 4 weeks period. The patients' clinical and demographic parameters, blood pressure, body weight, height and BMI were recorded before the study. Blood pressure measurement was performed before taking any morning doses of antihypertensive medicine, no less than 1 hour after exercising, smoking or consuming caffeine, after allowing for about 10 min to adjust to the temperature in

the examining room. During measurement, the patients' arm relaxed, uncovered and supported at the level of the heart. At 0 and 4 weeks, the subjects were weighed after an overnight fast, while they were wearing light indoor clothing and no shoes. Urine samples were also analyzed for the presence of glucose and infection indicators. Blood samples were taken at the first arrival and at the end of the study from fasting subjects for all biochemical analyses. At each occasion, a 10-mL serum tube was used. To obtain serum, the tube was left for ≥1 h after venipuncture at room temperature. Serum was prepared by centrifuging the tube at 4°C. In all serum samples, glucose, total cholesterol, HDL, LDL, VLDL cholesterol, triglyceride, HbA1c, and CRP were measured in addition to cholesterol/HDL ratio.

## 2.14. Statistical Analyses

Statistical analyses were performed by using SPSS 16 for Windows. Each result is expressed as the means±SEM for independent determinations. Differences between data were assessed by pair t test with the P value of <0.05.

## 3. Results and Discussions

The physicochemical analyses of Hawthorn vinegar produced in Bolu province revealed that the vinegar samples had a pH value of 3.28±0.56, TA of 3.7±0.3 g/100 mL acetic acid equivalence, °Brix of 5.33±0.4, conductivity of 3.86±0.45 mS/cm, total phenolic substance concentration of 5.02±0.23 mg/100 mL, total monomeric anthocyanin content of 0.51±0.34 mg/mL, total antioxidant capacity of 76.27±4.6 % and color (L\*, a\* and b\*) values of 31.4±5.2, 20.48±3.7 and 40.08±4.87, respectively.

It was revealed that the amount of phenolic compounds were detected as 17.5±2.3 mg/100 mL PC-Bz, 33.46±2.4 mg/100 mL EC, 13.45±3.2 mg/100 mL ChA, 2.7±0.03 mg/100 mL HP, and 4.10±0.4 mg/100 mL IQ, respectively. Results presented that Hawthorn vinegar has very high amount of total phenolic substance concentration, total monomeric anthocyanin content and total antioxidant capacity that have biological effects including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory actions [15,16]. The ability of some phenolic compounds to act as antioxidants has been demonstrated in the literature. Several researchers have investigated the antioxidative activity of flavonoid compounds and have attempted to define the structural characteristics of flavonoids that contribute to their activity [17,18,19]. Antioxidant activity is a fundamental property important for life. Many of the biological functions, such as antimutagenicity, anticarcinogenicity, and antiaging, among others, originate from this property [15,20,21]. From this standpoint it was claimed that Hawthorn vinegar has very important functional properties for health.

The amount of K, Ca, Mg, Fe, Na, Zn and Cu in hawthorn vinegar was detected as, 6638.7 ± 452.7, 521.4 ± 39.5, 241.9 ± 43.7, 240.0 ± 28.3, 123.1 ± 21.3, 40.1 ± 9.8 and 0.273 ± 0.5 ppm, respectively. The amount of these metal ions in fresh hawthorn fruit was detected as 4397.0±104.3, 2032.7±59.6, 654.7±54.7, 27.8±8.7,

1082.9±30.5, 4.9±0.5 and 0.43±0.0, respectively. Another study performed with hawthorn fruit grown in middle Anatolian part of Turkey reported that the amount of Ca, K, Mg, Na and P were 3046.37, 13531.96, 1502.55, 312.18 and 1477.88 ppm, consequently [22]. It was revealed with this study that hawthorn vinegar is very rich in the amount of K, Ca, Mg, Fe, Na and Zn.

Initial number of *L. monocytogenes* cells in control samples was detected as  $7.75 \pm 0.44$  log cfu/mL. With the addition of hawthorn vinegar at 5, 20, 40, 80, 160 and 320 µL/mL concentrations, the number of survived cells decreased to  $7.03 \pm 0.88$ ,  $6.00 \pm 0.39$ ,  $5.48 \pm 0.69$ ,  $4.80 \pm 0.34$ ,  $4.40 \pm 0.14$  and  $3.37 \pm 0.53$  log cfu/mL. Increased concentration of hawthorn vinegar caused a significant increase in the inactivation of *L. monocytogenes* ( $p \leq 0.05$ )

(Figure 1). Similarly, hawthorn vinegar caused a significant decrease in the initial *E. coli* O157:H7 cells. Number of *E. coli* O157:H7 cells ( $7.19 \pm 0.48$  log cfu/mL) transferred into test tubes decreased to  $6.83 \pm 0.50$ ,  $6.24 \pm 0.50$ ,  $5.92 \pm 0.39$ ,  $5.25 \pm 0.35$ ,  $4.49 \pm 0.48$  and  $2.30 \pm 0.38$  log cfu/mL with 5, 20, 40, 80, 160 and 320 µL/mL hawthorn vinegar concentrations, consequently (Figure 1). Numbers of *C. lipolitica* cells ( $6.22 \pm 0.41$  log cfu/mL) with the same concentrations of hawthorn vinegar were enumerated as  $5.95 \pm 0.26$ ,  $5.43 \pm 0.51$ ,  $5.23 \pm 0.58$ ,  $5.14 \pm 0.71$ ,  $4.64 \pm 1.12$  and  $3.88 \pm 1.65$  after the addition of hawthorn vinegar in the same concentrations (Figure 1). Yeast cells are more resistant to high acidity than bacteria, thus inactivation obtained in *C. lipolitica* cells was less than that of both *L. monocytogenes* and *E. coli* O157:H7.

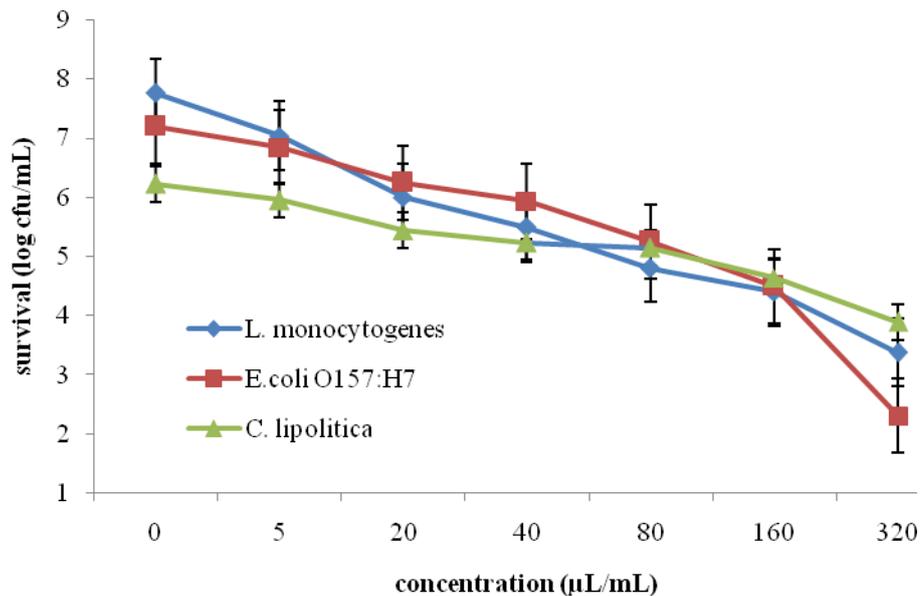


Figure 1. Inactivation of microorganisms by hawthorn vinegar

All the volunteers completed the study for the determination of metabolic effect; however, due to the distinct flavor and taste of the vinegar all patients expressed unwillingness to drink the vinegar especially at the last week or last days of the study. Total of 21 females with the mean age of  $62.76 \pm 2.0$  years and 16 males with mean age of  $56.31 \pm 2.4$  years were included in the study. Mean weight for female and male subjects, at the beginning of the study, was measured as  $78.35 \pm 2.47$  and  $85.68 \pm 3.30$  kg with body mass index (BMI) of  $31.47 \pm 0.7$  and  $29.80 \pm 0.8$  kg/m<sup>2</sup>, respectively. The mean weight and BMI index for female and male volunteers were recorded as  $77.36 \pm 2.4$  and  $84.02 \pm 3.0$  kg and  $31.06 \pm 0.7$  and  $29.23 \pm 0.8$  kg/m<sup>2</sup>, consequently at the end of the study. The weight loss and BMI decrease of the patients after four weeks vinegar supplementation had a significant difference with the mean weight loss of  $1.45 \pm 0.44$  kg in men and  $1.25 \pm 0.28$  kg in women. In addition to weight and the BMI, pulse rate of the subjects was also measured. Initial pulse rate for female and male subjects were measured as  $74.57 \pm 2.59$  and  $74.31 \pm 2.57$  beat/min and these values changed to  $71.90 \pm 2.49$  and  $72.68 \pm 2.00$  beat/min, respectively.

Systolic blood pressure before the study for female and male subjects was  $141.00 \pm 4.16$  and  $139.37 \pm 5.34$  mmHg before the study, and it was changed to  $130.90 \pm 4.39$  and  $128.12 \pm 4.20$  mmHg after the consumption of hawthorn

vinegar. A significant difference was detected for the diastolic blood pressure for female and male subjects before ( $83.90 \pm 1.96$  and  $78.37 \pm 3.54$  mmHg) and after the study ( $74.28 \pm 1.99$  and  $71.25 \pm 2.75$  mmHg).

The mean venous plasma glucose level for female subject decreased from  $150.42 \pm 12.02$  to  $121.76 \pm 5.94$  mg/dL, and it was decreased from  $177.18 \pm 17.98$  to  $153.87 \pm 18.12$  mg/dL for the male subjects ( $p < 0.001$ ). Similarly a significant decrease was also obtained in total cholesterol level. Cholesterol level in female and male subjects at the beginning of the study was  $197.23 \pm 7.05$  and  $198.75 \pm 6.63$  mg/dL, and these values decreased to  $189.04 \pm 5.39$  and  $186.25 \pm 7.27$  mg/dL, respectively. Triglyceride level of the female patients decreased from  $180.04 \pm 7.89$  to  $168.19 \pm 6.94$  mg/dL. The triglyceride level of the male patients also showed a great decrease, and it reduced from  $260.68 \pm 11.45$  to  $238.31 \pm 17.48$  mg/dL. LDL level for female and male subjects changed from  $116.56 \pm 9.45$  and  $118.25 \pm 12.30$  mg/dL to  $113.71 \pm 28.20$  and  $115.01 \pm 12.86$  mg/dL. HDL level from female and male patients decreased from  $44.10 \pm 1.62$  and  $33.67 \pm 1.37$  to  $41.33 \pm 1.55$  and  $36.68 \pm 1.37$  mg/dL. The ratio of cholesterol to HDL level (KOLHDL) at the beginning of the study for female and male subjects changed from  $4.94 \pm 0.32$  and  $5.68 \pm 0.54$  to  $4.64 \pm 0.22$  and  $5.09 \pm 0.57$ . VLDL level of female patients at the beginning of the study measured as  $37.96 \pm 1.84$  reduced to  $36.87 \pm 2.52$

mg/dL. The same value for male patients changed from  $39.67 \pm 4.42$  to  $37.03 \pm 4.07$  mg/dL. Female subjects had the HbA1c level of  $6.92 \pm 0.26$  and it changed to  $8.01 \pm 0.45$  % at the end of the study. HbA1c level for the male patients changed from  $8.01 \pm 0.45$  to  $7.80 \pm 0.42$  %, consequently. CRP levels for the female and the male patients at the beginning of the study was  $7.03 \pm 1.25$  and  $6.13 \pm 0.97$  mg/L,

and these values reduced to  $6.05 \pm 1.15$  and  $5.36 \pm 0.90$  mg/L at the end of the study. When these parameters were compared as before and after the study with taking the average of whole patients, there was a significant difference on weight, BMI, sys blood pressure, diastolic blood pressure, glucose, HbA1c, cholesterol, triglyceride, LDL, HDL, total cholesterol/HDL and CRP levels (Table 1).

**Table 1. Clinical and biochemical factors changes after the regular vinegar intake in patients with high cardiovascular risk group**

Biochemical and Clinical Parameters	Before the study	After the study	P Value
Weight (kg)	$81.52 \pm 2.06$	$80.24 \pm 1.99$	<.0001
BMI (kg/m <sup>2</sup> )	$31.4 \pm 0.57$	$30.27 \pm 0.57$	<.0001
Pulse rate (beat/min)	$74.45 \pm 1.82$	$72.24 \pm 1.64$	NS
Sys Blood Pressure (mmHg)	$140.29 \pm 3.26$	$129.70 \pm 3.05$	<.0001
Diastolic Blood Pressure (mmHg)	$81.51 \pm 1.92$	$72.97 \pm 1.63$	<.0001
Glucose (mg/dL)	$162 \pm 10.4$	$135.6 \pm 8.8$	<.001
HbA1c (%)	$7.39 \pm 0.25$	$7.12 \pm 0.24$	<.001
Cholesterol (mg/dL)	$197.9 \pm 8.2$	$187.8 \pm 7.7$	<.005
Triglyceride (mg/dL)	$214.9 \pm 19.8$	$198.5 \pm 18.2$	<.05
VLDL mg/dL	$38.61 \pm 2.0$	$36.9 \pm 2.16$	NS
LDL mg/dL	$117.29 \pm 7.44$	$112.67 \pm 6.66$	<.0001
HDL mg/dL	$39.8 \pm 1.4$	$41.1 \pm 1.2$	=.051
Total Cholesterol/ HDL	$5.13 \pm 0.25$	$4.65 \pm 0.19$	<.001
CRP mg/L	$6.65 \pm 0.83$	$5.76 \pm 0.7$	<.0001

There was no significant difference between the gender according to all parameters except for HDL values, Cholesterol/HDL ratios and HbA1c values. The mean HDL cholesterol value at the beginning of the study was  $44.81 \pm 1.62$  mg/dL in women and  $33.95 \pm 1.45$  mg/dL in men ( $p < 0.0001$ ). The values increased to  $45.15 \pm 1.38$  mg/dL in women and  $36.17 \pm 1.39$  mg/dL in men

( $p < 0.0001$ ). The mean cholesterol/HDL ratio was 4.51 in women and 5.85 in men ( $p < 0.05$ ), and these values decreased to 4.27 in women and 5.02 in men ( $p = 0.053$ ). The mean HbA1c values was  $6.78 \pm 0.23$  % in women and  $8.11 \pm 0.43$  % in men ( $p < 0.005$ ), and the values decreased to  $6.49 \pm 0.18$  % in women and  $7.86 \pm 0.40$  % in men ( $p < 0.005$ ) (Table 2).

**Table 2. The only significant biochemical differences between male and female groups**

Biochemical Parameters	Women	Men	P Value
After the study HDL mg/dL	$44.81 \pm 1.62$	$33.95 \pm 1.45$	<.0001
Before the study HDL mg/dL	$45.15 \pm 1.38$	$36.17 \pm 1.39$	<.0001
After the study cholesterol/HDL	$4.51 \pm 0.24$	$5.85 \pm 0.41$	<.05
Before the study cholesterol/HDL	$4.27 \pm 0.21$	$5.02 \pm 0.31$	=.053
After the study HbA1c(%)	$6.78 \pm 0.23$	$8.11 \pm 0.43$	<.005
Before the study HbA1c(%)	$6.49 \pm 0.18$	$7.86 \pm 0.40$	<.005

Due to the limited amount of hawthorn vinegar production, it was not possible to compare the results obtained in this study with previous studies, but hawthorn fruit extract has been shown to have many health benefits including being cardiovascular protective, hypotensive and hypocholesterolemic. Supplementation of 2% hawthorn fruit powder significantly elevated serum  $\alpha$ -tocopherol by 18–20% in rats fed with 30% polyunsaturated canola oil diet, as compared with the control [23,24]. The present results suggest that part of the mechanism for cardiovascular protective effects of hawthorn fruit might also involve the direct protection to human LDL from oxidation or indirect protection via maintaining the concentration of  $\alpha$ -tocopherol in human LDL.

The rapid increase in obesity and diseases related to the insulin resistance syndrome is a growing public health problem. The health costs of metabolic disorders will rise profoundly, and it is a great challenge for the future to find means to counteract this development. The quality of the diet has been shown to play an important role in the combat of metabolic disorders. At this time point, there is a substantial amount of evidence that a diet characterized by a low glycemic index has benefits in both prevention

and treatment of several diseases linked to the insulin resistance syndrome, such as cardiovascular diseases and type II diabetes [25].

Vinegar which is used commonly as a condiment has been proven to have some medical uses as well. Acetic acid is the main component of vinegar. Some other constituents include, anthocyanin (e.g. cyanidin-3-glucoside) flavonols (e.g. quercetin, kaempferol), flavanols (catechin, epicatechin) [26], vitamins, mineral salts, amino acids and nonvolatile organic acids (eg. tartaric, citric, malic, lactic) [27,28,29]. Vinegar has shown such multiple effects as enhancement of glycogen repletion [28,29], prevention of hypertension [29], stimulation of Ca<sup>+2</sup> absorption [30] and reduction serum total cholesterol and triacylglycerol in animal studies [30]. Many recent studies have documented that vinegar ingestion decreases the glucose response to a carbohydrate load in normal and diabetic subjects [2,3]. This study suggests that vinegar might have some acute effects on biochemical risk factors of atherosclerosis, and a probable protective value can be considered for its postprandial use. All these data suggest a probable protective value for vinegar. Considering that vinegar is a safe product, widely available and affordable,

it is possible to use it for the treatment of biochemical risk factors of atherosclerosis.

Aqueous alcohol extracts of hawthorn fruits and leaves are used as dietary supplements and herbal medicines in the United States and Europe for treating heart failure degrees I-III according to the classification of the New York Heart Association (NYHA) [32]. It has been claimed that hawthorn fruit is capable of reducing food stagnancy, stasis, blood lipids and blood pressure [33,34,35,36].

The present study clearly demonstrated that hawthorn vinegar possessed very strong metabolic effects in patients with high cardiovascular risk. Supplementation of hawthorn vinegar lowered body weight, blood pressure, serum glucose, HbA1c, cholesterol, LDL and triglyceride level. At the same time, significant HDL increase and total cholesterol/HDL decrease were recorded in the study.

In conclusion, regular hawthorn vinegar usage have some beneficial effects on clinical and biochemical risk factors of atherosclerosis and a probable protective value can be considered for its postprandial use. It is possible that high total phenolic substance concentration may be responsible some of the metabolic effects. Thus, the effect of individual phenolic substances needs to be searched to evaluate the full potential of hawthorn vinegar on human health.

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## Statement of Competing Interests

The authors have no competing interests.

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