

# Evaluation of the effects of a honey-based gel on blood redox biomarkers and the physiological profile of healthy adults: A pilot study

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**Abstract.** Honey is a natural product derived from the insect *Apis mellifera*. Approximately 200 different compounds are included, making it a complex mixture with antimicrobial, antioxidant, and antidiabetic activity. Flavonoids and phenolic acids contained in honey are associated with its antioxidant capacity via mechanisms such as hydrogen donation and metallic ion chelation, although the exact antioxidant mechanism remains unknown. The aim of the present study was to: i) Estimate the antioxidant activity of a natural honey-based gel, commercially available under the trade name of 'Bear Strength honey gel' and to ii) assess the physiological and redox adjustments obtained after its consumption in healthy adult participants. For this purpose, 20 healthy participants (10 men and 10 women) included in their habitual diet 70 g of the honey-based gel for 14 days in a row. Pre- and post-consumption, physiological [weight, height, body mass index, body fat, waist-to-hip ratio, resting heart rate and blood pressure (BP)] and hematological (complete blood count) data

were evaluated, along with the levels of five redox biomarkers: Glutathione (GSH), catalase (CAT), total antioxidant capacity (TAC), protein carbonyls (PCARBS) and thiobarbituric reactive substances (TBARS). The results revealed that the honey-based gel decreased the diastolic and mean arterial BP, especially in women, without affecting the rest of the physiological and hematological variables. Regarding the changes observed in antioxidant status variables, GSH was increased both in the total and women's group, while TAC was increased in all groups post-consumption. No changes were detected in the levels of CAT. Regarding oxidative stress, a decrease in the levels of TBARS in the total and women's group, was observed. PCARBS levels were decreased post-consumption only in the women's group. In conclusion, the present study demonstrated the potential positive effects of a honey-based gel on BP and redox status of healthy adults in a sex-specific manner.

## Introduction

Research on the consumption of functional foods over the last decades has established their importance for the promotion of optimal human health. Importantly, dietary antioxidants hold a significant role in the prevention of reactive oxygen species formation or even in scavenging free radicals through radical chain reaction interruption, molecules that are responsible for triggering numerous pathological conditions and diseases (1). Previous studies have elaborated on the chemo-preventive properties of natural food products (2,3), a large number of which have examined the nutritional importance of honey (4-6). Honey is a natural product derived from the insect *Apis mellifera*, which holds a significant role in the field of agriculture (7). These insects serve as plant and crop species pollinators to enhance the biodiversity of agricultural and also non-agricultural landscapes (1). The use of honey has been recorded from ancient civilizations, not only as a food but also as a medicine for relieving gastrointestinal disorders (8) and wound healing by reducing the oedema, inflammation, and exudation that frequently develop in numerous types of wounds (8,9).

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**Abbreviations:** GSH, glutathione; GST, glutathione S-transferase; CAT, catalase; MDA, malondialdehyde; TPC, total phenolic content; FC, Folin-Ciocalteu; RT, room temperature; GAEs, gallic acid equivalents; RSC, radical scavenging; HRP, horseradish peroxidase; WHR, waist-to-hip ratio; BP, blood pressure; CBC, complete blood count; RBCL, red blood cell lysate; TCA, trichloroacetic acid; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; TBA, thiobarbituric acid; DNPH, 2,4-dinitrophenylhydrazine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; PCARBS, protein carbonyls

**Key words:** honey, oxidative stress, antioxidant activity, blood pressure, body composition, functional food

Nectar selection from different plant species, represents the main factor determining the different types of honey, resulting in a wide variation in its colour, taste, texture and, composition (10-12). Honey has a complex composition, containing ~200 compounds such as sugars, proteins, vitamins, water, free amino acids, enzymes, minerals, and numerous phytochemicals (13). Due to its composition, enriched with many bioactive ingredients, several studies have established its antimicrobial (14), antiviral (15), anticancer (16), antidiabetic (17) and antioxidant (18) properties. This multilayer activity of honey has been suggested for the protection against pathologies interrelated with the cardiovascular (19), nervous (20), respiratory, and gastrointestinal system (21).

Oxidative stress has been revealed as the main contributing factor in several pathologies, initiating structure modifications and function modulations in nucleic acids, lipids, and proteins (22). Specifically, it has been associated with several pathological conditions such as neurodegenerative disorders (Alzheimer, Parkinson, etc.) (23), cancer (16), diabetes obesity, and cardiovascular diseases (24,25). Previous animal studies have demonstrated the relationship between honey and oxidative stress-induced conditions. Specifically, Abdulmajeed *et al* concluded that honey consumption was able to increase glutathione (GSH) and glutathione S-transferase (GST) levels in brain tissue of rats after they were exposed to lead for 28 days (26). Moreover, honey appears to have the ability to enhance the activity of major antioxidant enzymes such as superoxide dismutase (SOD), glutathione-disulfide reductase (GR), catalase (CAT) and glutathione peroxidase (GPx) in the liver of rats after they consumed acetaminophen for 10 days (27). Despite the strong evidence suggesting the chemo-preventive role of honey, it is still under debate which specific ingredients of honey and in which composition, are liable for its activity (28). It is common knowledge that honey composition is mainly affected by botanical origin, however, geographical factors can also influence its synthesis and consequently its quality (29). Constituents such as glucose oxidase, catalase, organic acids, amino acids, proteins, phenolic acids, and flavonoids have been suggested to play a significant role in the antioxidant capacity of honey (30). Apart from the components aforementioned, polyphenols contained in honey have been proposed to affect its antioxidant capacity (31). Even though the antioxidant capacity of honey is well documented, the exact antioxidant mechanism is still unknown (32). Some of the most studied interrelated mechanisms include, but are not limited to, hydrogen donation, metallic ion chelation and scavenging of free radicals by increasing the endogenous levels of important antioxidant molecules and enzymes such as  $\beta$ -carotene vitamin C, glutathione reductase and uric acid (32). *In vitro* studies regarding honey are predominant in the literature (33-37), but *in vivo* studies, especially concerning the metabolic changes caused in humans are scarce. Schramm *et al*, recruited 40 healthy human volunteers to test the impact of honey consumption on the antioxidant and reducing capacity in plasma. The research indicated that consumption of 1.5 g honey/kg of body weight had a positive effect on their antioxidant and reducing capacity, supporting the concept that honey consumption may have a positive impact on the antioxidant defense system of healthy human subjects (38). Moreover, another human clinical

trial examined the effects of a honey, rich in probiotics, on 60 patients suffering from diabetic nephropathy (39). In this study, patients consumed 25 g of honey rich in probiotics per day for 12 weeks and malondialdehyde (MDA) levels were found to be significantly lower following the consumption of the honey rich in probiotics (39). The effects of specific types of honey on physiological parameters have shown that consumption of 20 g/day of Tualang honey, for 12 months resulted in significant decreases in diastolic blood pressure (BP) and fasting blood glucose in postmenopausal women (40).

To this end, honey has been proposed as the 'gold treasure' with health-promoting effects against oxidative stress-related dysfunctions. However, the effects of consumption of a novel natural honey-based gel on redox biomarkers, blood chemistry and physiological characteristics have not been investigated in healthy individuals. Therefore, the present pilot study aimed: i) To estimate the antioxidant potential of a honey-based gel and ii) to monitor the physiological redox and adjustments obtained after its consumption in healthy participants, and iii) to assess whether the responses of the assessed variables would be different between men and women.

## Materials and methods

**Study design.** A total number of 20 participants took part in the present study, and were separated into two equal subgroups of 10 individuals, as a preliminary power analysis (a probability error of 0.05, and a statistical power of 80%) showed that this sample size per group was the appropriate, in order to detect statistically meaningful changes between groups. The division in two groups was performed according to sex, therefore, 10 men and 10 women with a mean age of  $39.0 \pm 11.5$  years (range, 22-52 years) were recruited for this clinical study. The criteria defined for the participants included: i) Absence of musculoskeletal injury and cardiovascular/metabolic disease; ii) not consuming nutritional supplements and medication prior to ( $\geq 6$  months) and during the study; and iii) non-smoking. Following recruitment, volunteers completed a health history questionnaire and provided their written consent for participation after they were informed about the purpose, methodology and possible risks associated with the study. Participants received 70 g of a honey-based gel, commercially available under the trade name of 'Bear Strength honey gel' (Nomad Premium Greek Honey), per day for 14 consecutive days. The honey-based gel is a natural product containing 87% fir honey, and pollen. Prior to its consumption and 14 days post-consumption, participants provided a blood sample and underwent an assessment of their physiological profile. Along with the honey-based gel consumption, no other restrictions or limitations were set regarding food intake. Dietary intake was recorded for 3 days prior to first blood sample collection and participants were instructed to follow the same diet prior to second blood collection. Furthermore, participants were also instructed to abstain from any strenuous physical activity, prior to pre- and post-blood sampling and physiological profile assessment, to avoid possible exercise-related alterations of their redox profile (41). The first blood samples collected from the participants prior to the honey-based gel consumption was defined as the control group. The Institutional Review Board of the Department of Physical Education and Sport Science

of the University of Thessaly (protocol ID: 1722/9-12-2020) approved the methods, procedures, and ethics of this study. Procedures agreed with the 1975 Declaration of Helsinki as revised in 2013.

**Total phenolic content (TPC) of the honey-based gel.** The TPC value of the honey-based gel sample was estimated using Folin-Ciocalteu (FC) reagent. In brief, 20  $\mu$ l of the sample, 1 ml of dH<sub>2</sub>O and 100  $\mu$ l of FC reagent were agitated and incubated for 3 min, in the dark at room temperature (RT). Next, 25%w/v Na<sub>2</sub>CO<sub>3</sub> (280  $\mu$ l) and dH<sub>2</sub>O (600  $\mu$ l) were vortexed and incubated for 60 min, RT, in the dark. Finally, the absorbance was measured at 765 nm (Hitachi, U 1900 UV/VIS; Hitachi High Technologies Corporation). The absorbance value of a blank was deducted from the absorbance value of the tested sample, where the blank contained the FC reagent and dH<sub>2</sub>O. Increasing concentrations of gallic acid (0, 50, 150, 250 and 500  $\mu$ g/ml) were used to create a standard curve for TPC value estimation, which was expressed as mg of gallic acid equivalents (GAEs) per g of honey (mg GAE/g honey) (42).

**ABTS<sup>•+</sup> radical scavenging (RSC) assay.** The ABTS<sup>•+</sup> scavenging capacity of the honey-based gel was determined according to Miller *et al* (43) with some modifications (44). Briefly, 400  $\mu$ l of dH<sub>2</sub>O, 1 mM of ABTS<sup>•+</sup> solution (500  $\mu$ l), 30  $\mu$ M hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (50  $\mu$ l) and 6  $\mu$ M horseradish peroxidase (HRP) (50  $\mu$ l) were mixed and then incubated for 45 min in the absence of light at RT. Subsequently, 50  $\mu$ l of each dilution (25-0.78 mg/ml) were added and the absorbance was measured at 730 nm using a spectrophotometer (Hitachi, U 1900 UV/VIS). As a blank, an ABTS<sup>•+</sup> radical solution without the addition of the enzyme (HRP) was used, while the mixture in the absence of the tested sample was used as a control. The results were interpreted as follows:

$$\text{RSC (\%)} = \frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{control}}}{\text{OD}_{\text{control}}} \times 100,$$

where OD<sub>control</sub> and OD<sub>sample</sub> are the absorbance values of the control and the sample, respectively. The capacity of the honey-based gel to scavenge the free radicals was estimated through a half maximal inhibitory concentration value (IC<sub>50</sub>), which was determined from the graph-plotted percentage against the sample concentration.

**Physiological characteristics.** A stadiometer (Beam Balance Stadiometer 208; Seca United Kingdom) was used in order to measure the body mass and the height (to the nearest 0.05 kg and 0.1 cm) of the participants. During measurements, participants were barefoot and lightly dressed (45). Waist and hip circumferences were obtained with a measuring tape. Waist-to-hip ratio (WHR) was calculated by the use of the equation: WHR = waist circumference/hip circumference. Body fat percentage was measured by dual-energy X-ray absorptiometry (Lunar DPX NT; GE Healthcare) as previously described (46). Resting heart rate (RHR) was measured using a heart rate monitor (Polar Electro) with participants in the supine position and following a 5-min rest as previously described (47). Systolic and diastolic BP were assessed by a physician using an arm sphygmomanometer (Precisa N R-1362; Rudolf Riester GmbH) according to a standardized procedure established by the American Heart Association (48). Briefly, participants had to be seated

and rested for 5 min prior to the exam. Systolic and diastolic BP was measured according to the first and fifth Korotkoff sounds and each reading was performed in duplicate (with a 1-min break between readings) (49). Participants were asked whether they had any difficulty in consuming the product or there were any side effects from the honey-based gel intake.

**Blood collection.** In EDTA tubes, 10 ml of blood were collected, and a small portion was analyzed for the complete blood count (CBC) parameters using an automatic hematology analyzer (Mythic 18 Orphee; Orphee-Medical; Cormay Diagnostics). The remaining blood was centrifuged at 1,370 x g for 10 min at 4°C and the upper layer (plasma) was isolated. The total amount of erythrocytes was diluted in water (1% v/v), followed by centrifugation at 4,000 x g for 15 min at 4°C and the supernatant was collected. Both red blood cell lysate (RBCL) and plasma samples were stored at -20°C until use (50).

**Determination of hemoglobin concentration.** A commercial kit (Hemoglobin kit; article no. 60230; Dutch Diagnostics BV) was used in order to assess the hemoglobin levels, according to manufacturer's instructions. Briefly, 5  $\mu$ l of RBCL were mixed with 1 ml of hemoglobin reagent (Reagent R1, pH 7.3). After a 10-min incubation in the absence of light and at RT, the optical density was measured at 540 nm (Hitachi, UV-1900). As a blank, 1 ml of the reagent (R1) was used.

**Determination of reduced GSH levels.** A total of 400  $\mu$ l of RBCL was precipitated with 400  $\mu$ l 5% (w/v) TCA, followed by centrifugation at 15,000 x g for 5 min at 4°C. This step was repeated and 90  $\mu$ l of 5% (w/v) TCA were added to 300  $\mu$ l of the supernatant, followed by centrifugation as aforementioned. GSH levels were quantified according to a study by Reddy *et al* (51), with some alterations (52). Briefly, 20  $\mu$ l of clarified RBCL was dissolved in 67 mmol/l (660  $\mu$ l) potassium sodium phosphate (pH 8.0) and 1 mmol/l (330  $\mu$ l) 5,5'-dithiobis-2-nitrobenzoic acid followed by incubation at RT for 10 min, in the absence of light. The optical density was measured at 412 nm (Hitachi, UV-1900) in order to interpret the results with a calibration curve using the millimolar extinction coefficient of 2-nitro-5-thiobenzoate (13.6 l/mmol/cm) as previously reported (52).

**Catalase activity.** Catalase activity was estimated according to a method described in a study by Aebi (53), which was slightly revised (54). A 1:10 dilution of RBCL (4  $\mu$ l) was added to 2,996 ml of 67 mmol/l sodium potassium phosphate (pH 7.4) and incubated for 10 min at 37°C. Subsequently, 5  $\mu$ l of 30% H<sub>2</sub>O<sub>2</sub> were added in the cuvette that was placed in the spectrophotometer (Hitachi, UV-1900) and the optical density was measured twice, at time point 0 and 120 sec, at 240 nm. The calculation of catalase activity was based on the molar extinction coefficient of H<sub>2</sub>O<sub>2</sub> (43.6 M<sup>-1</sup> cm<sup>-1</sup>) as previously reported (54).

**Determination of total antioxidant capacity (TAC).** TAC was performed as described in a previous study (55). To 10 mmol/l sodium potassium phosphate pH 7.4 (480  $\mu$ l) and 0.1 mmol 2,2-diphenyl-1-picrylhydrazyl (DPPH)<sup>•</sup> (500  $\mu$ l), 20  $\mu$ l of plasma were added and incubated at RT for 1 h, in the absence

Table I. Assessment of physiological characteristics following consumption of a honey-based gel.

Group Parameter	Total		Women		Men	
	Pre	Post	Pre	Post	Pre	Post
Body Weight (kg)	76.82±4.02	77.1±4.15	61.65±2.15	61.5±2.14	91.99±3.46	92.7±3.49
Height (m)	1.73±0.02	1.73±0.02	1.66±0.02	1.66±0.02	1.80±0.02	1.80±0.02
BMI (kg/m <sup>2</sup> )	25.34±0.98	25.42±1.00	22.39±0.88	22.33±0.87	28.29±1.07	28.51±1.07
Body fat (%)	27.28±1.24	27.65±1.22	28.95±1.61	29.1±1.69	25.61±1.64	26.21±1.55
Waist Circumference (cm)	84.82±3.02	84.60±3.14	73.50±1.67	72.90±1.79	96.15±2.59	96.30±2.64
Hip Circumference (cm)	104.20±1.72	104.30±1.74	99.20±1.50	99.20±1.56	109.30±1.99	109.50±1.96
WHR (ratio)	0.81±0.02	0.81±0.02	0.74±0.01	0.73±0.01	0.88±0.01	0.88±0.01
Systolic BP (mm Hg)	115.70±2.25	111.20±2.52	112.70±4.03	105.00±3.24	118.80±1.11	117.40±2.47
Diastolic BP (mm Hg)	79.10±2.05	74.75±2.52 <sup>a</sup>	75.70±2.87	68.70±2.92 <sup>a</sup>	82.50±2.34	80.80±2.88
Resting HR (b/min)	63.85±1.49	62.20±1.7	64.70±1.69	64.70±1.92	63.00±2.33	59.70±2.48
Mean arterial BP (mm Hg)	91.30±2.12	86.88±2.52 <sup>a</sup>	88.02±3	80.79±2.91 <sup>a</sup>	94.58±2.83	92.99±2.44

<sup>a</sup>P<0.05, a significant difference from the pre value of the same group. BMI, body mass index; WHR, waist-to-hip ratio; BP, blood pressure; HR, heart rate.

of light. At the end of the incubation period, centrifugation was performed at RT for 3 min at 15,000 x g, and the optical density was measured at 520 nm (Hitachi, UV-1900). The results were estimated by the reduction of DPPH to 2,2-diphenyl-1-picrylhydrazine (DPPH:H) caused by the plasma antioxidants, and were presented as mmol DPPH/l plasma.

*Thiobarbituric acid reactive substances (TBARS).* TBARS levels were identified according to a previously described protocol (56) slightly modified (52). Particularly, in a reaction tube containing 100  $\mu$ l of plasma, 500  $\mu$ l of 35% (w/v) TCA and 200 mM Tris-HCl buffer, pH 7.4 (500  $\mu$ l) were added and incubated for 10 min at RT. Subsequently, 1 ml of a 2-M Na<sub>2</sub>SO<sub>4</sub>-55 mM TBA solution was added, followed by incubation in a 95°C water bath for 45 min, and an additional incubation in ice for 5 min. Finally, 1 ml of 70% (w/v) TCA was combined with the samples, vortexed and 1 ml of each testing sample was transferred to a new centrifuge tube. A centrifugation at 11,200 x g, for 3 min at RT was performed and the optical density was determined at 530 nm (Hitachi, UV-1900). The results were based on the molar extinction coefficient of malondialdehyde (156x10<sup>3</sup> M<sup>-1</sup> cm<sup>-1</sup>) as previously reported (56).

*Protein carbonyls (PCARBS).* The determination of protein carbonyls was performed according to a study by Skaperda *et al.* (54). In centrifuge tubes, 50  $\mu$ l of plasma and 50  $\mu$ l of 20% (w/v) TCA were added, incubated for 15 min at RT and centrifuged at 15,000 x g for 5 min at 4°C. Subsequently, the supernatant was removed, and the pellet was resuspended in 10 mM 2,4-dinitrophenylhydrazine (DNPH) (500  $\mu$ l; dissolved in 2.5 N HCl). A blank was used for each sample, in which the pellet was resuspended in 2.5 N HCl (500  $\mu$ l) instead of DNPH. Samples and their respective blanks were both incubated at RT for 60 min in the absence of light, stirring every 15 min. After 1 h, the samples and blanks were centrifuged (15,000 x g, for 5 min, at 4°C), the supernatant was removed, the pellets were resuspended in 1 ml of 10% (w/v)

TCA and centrifuged as aforementioned. Subsequently, the pellets were washed in 50% v/v ethanol-ethyl acetate solution (1 ml) and centrifuged at 15,000 x g, for 5 min, at 4°C. The last step was repeated twice. Next, the pellets were resuspended in 5 M, urea pH 2.3 (1 ml) followed by a 15-min incubation at 37°C. Following centrifugation of the samples (15,000 x g, for 5 min, at 4°C), the optical density was monitored at 375 nm (Hitachi, UV-1900). The calculation of the protein carbonyl levels was based on the molar extinction coefficient of DNPH (22x10<sup>3</sup> M<sup>-1</sup> cm<sup>-1</sup>) as previously reported (54).

*Chemicals.* Chemicals used for all the aforementioned assays were supplied by Sigma-Aldrich; Merck KGaA.

*Statistical analysis.* Data normality was verified using the Shapiro-Wilk test. A 2x2 repeated measures ANOVA [group (men and women) by time (pre and post)] was used to identify possible sex-specific changes. If a significant interaction was detected, pairwise comparisons were performed through simple contrasts and simple main effects analysis using the LSD test method. Pre- and post-measurements within conditions were compared using paired t-tests. The statistical analysis of the results was performed using GraphPad Prism version 8.0.1 (for Windows; GraphPad Software, Inc.) and expressed as the mean  $\pm$  standard error of the mean (SEM). Each assay was performed in triplicate. P<0.05 was considered to indicate a statistically significant difference.

## Results

Participants did not report any side effects from the consumption of the honey-based gel.

The TPC of the honey-based gel was 129 mg GAE/100 g and, the IC<sub>50</sub> value of the ABTS<sup>+</sup> RSC assay was 0.88 mg/ml (data not shown).

Physiological and CBC data are presented in Tables I and II, respectively. The consumption of the honey-based gel resulted

Table II. Complete blood count responses following consumption of a honey-based gel.

Group Parameter	Total		Women		Men	
	Pre	Post	Pre	Post	Pre	Post
WBC ( $10^3/\mu\text{l}$ )	5.61±0.32	5.67±0.34	5.49±0.31	5.54±0.34	5.74±0.32	5.81±0.32
LYM (%)	36.19±1.65	35.96±1.55	37.42±1.79	36.7±1.96	34.96±1.29	35.23±1.73
MON (%)	4.56±0.49	4.05±0.46	4.71±0.58	3.38±0.30	4.42±0.34	4.73±0.48
GRA (%)	59.24±1.92	59.98±1.69	57.87±2.17	59.92±1.22	60.62±1.39	60.04±1.98
RBC ( $10^6/\mu\text{l}$ )	4.66±0.18	4.65±0.18	4.38±0.08	4.38±0.09	4.94±0.2	4.92±0.19
HGB (g/dl)	14.17±0.36	14.14±0.36	13.54±0.32	13.53±0.36	14.81±0.27	14.76±0.22
HCT (%)	40.82±0.89	40.44±0.89	39.46±0.86	39.03±0.95	42.19±0.63	41.85±0.46
MCV ( $\mu\text{m}^3$ )	88.28±2.39	87.64±2.39	90.09±1.34	89.14±1.53	86.47±2.89	86.14±2.84
MCH (pg)	30.68±0.97	30.65±0.92	30.96±0.69	30.91±0.61	30.41±1.14	30.40±1.10
MCHC (g/dl)	34.71±0.39	34.96±0.27	34.33±0.41	34.66±0.17	35.09±0.28	35.26±0.30
RDW (%)	13.46±0.44	13.60±0.37	13.17±0.29	13.35±0.30	13.76±0.52	13.85±0.39
PLT ( $10^3/\mu\text{l}$ )	268.70±17.00	272.08±19.65	276.20±14.11	263.30±17.22	261.30±18.4	282.30±20.45
MPV ( $\mu\text{m}^3$ )	7.92±0.21	8.12±0.21	7.82±0.14	8.19±0.27	8.02±0.25	8.05±0.24
PCT (%)	0.21±0.01	0.22±0.01	0.21±0.01	0.21±0.01	0.21±0.01	0.22±0.01
PDW (%)	14.89±0.47	15.39±0.59	14.4±0.36	15.36±0.48	15.39±0.49	15.43±0.65

WBC, white blood cells; LYM, lymphocytes; MON, monocytes; GRA, granulocytes; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; PLT, platelets; MPV, mean platelet volume; PCT, volume occupied by platelets in the blood as a percentage; PDW, platelet distribution width.

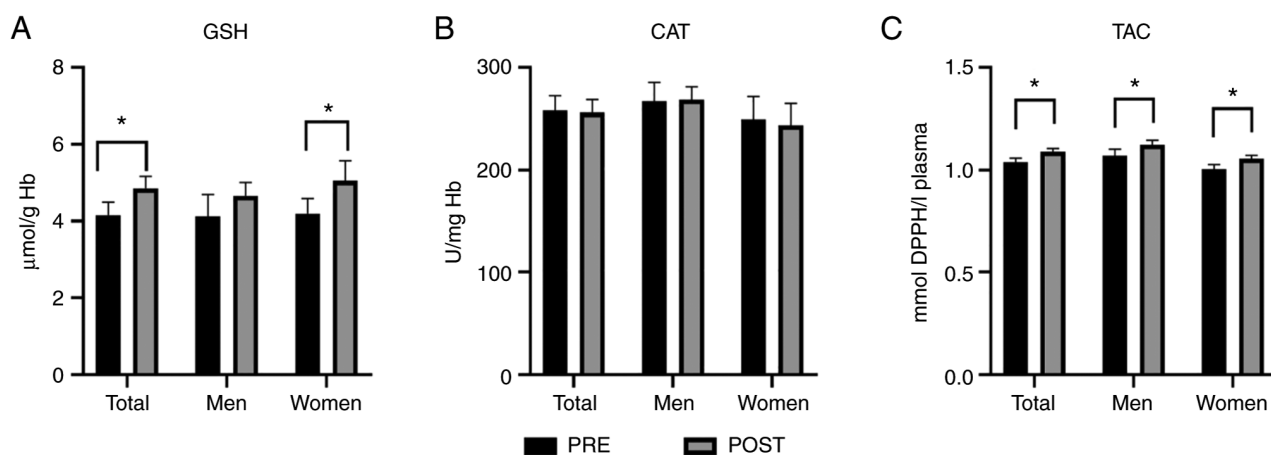


Figure 1. (A) Glutathione (B) catalase, and (C) total antioxidant capacity responses following consumption of the honey-based gel. All results are expressed as the mean  $\pm$  standard error of the mean. \* $P < 0.05$ , a significant difference from the pre value of the same group. GSH, glutathione; CAT, catalase; TAC, total antioxidant capacity; PRE, prior to consumption of the honey-based gel; POST, post consumption of the honey-based gel.

in significant decreases in diastolic and mean arterial BP when the data was combined (Table I). Comparison of the responses between the two sexes revealed changes after the consumption of the honey-based gel, resulting in significant decreases in women but not in men (Table I). There were no significant changes due to honey gel consumption in the other physiological and CBC measures (Table II).

The consumption of the honey-based gel resulted in significant increases in GSH and TAC when the data was combined (Fig. 1A and C, respectively). Comparison of the responses between the two sexes revealed changes after the consumption

of the honey-based gel in GSH levels, resulting in significant increases in women but not in men (Fig. 1A). There were no significant changes due to honey gel consumption in CAT (Fig. 1B).

The consumption of the honey-based gel resulted in significant decreases in TBARS when the data was combined (Fig. 2A). Comparison of the responses between the two sexes revealed changes after the consumption of the honey-based gel, resulting in a significant decrease in women but not in men (Fig. 2A). Furthermore, comparison of the responses between the two sexes with regard to PCARBS revealed changes after

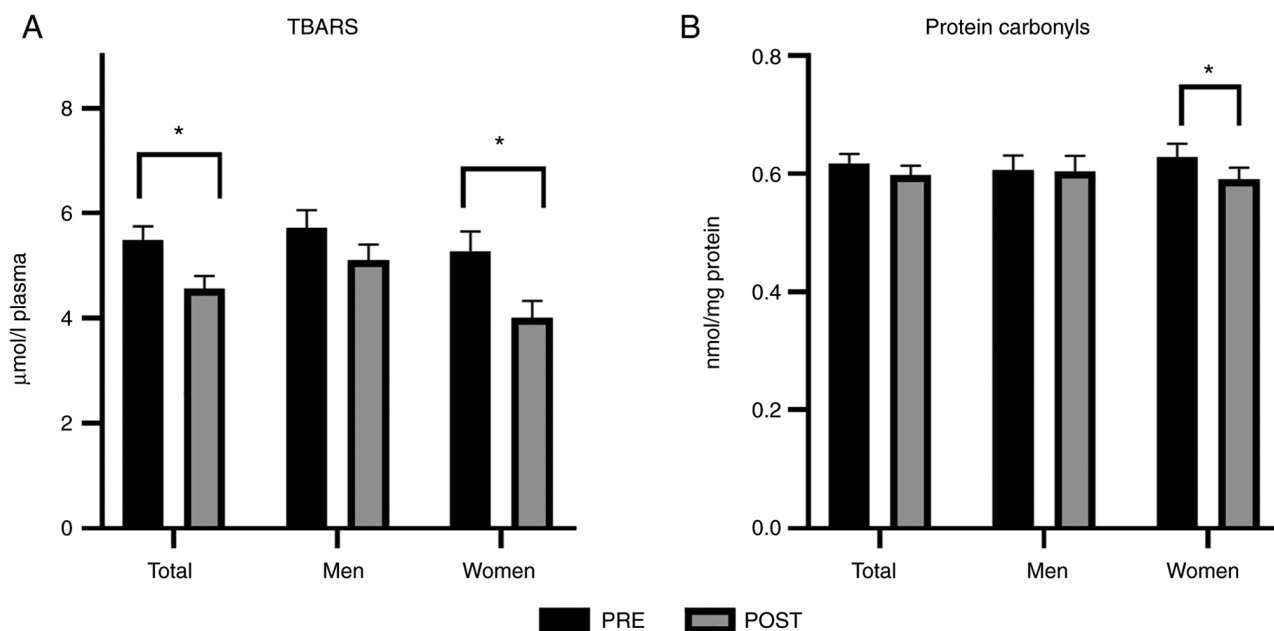


Figure 2. (A) Thiobarbituric reactive substances and, (B) protein carbonyls responses following consumption of the honey-based gel. All results are expressed as the mean  $\pm$  standard error of the mean. \* $P < 0.05$ , a significant difference from pre value of the same group. TBARS, thiobarbituric reactive substances; PRE, prior to consumption of the honey-based gel; POST, post consumption of the honey-based gel.

the consumption of the honey gel, resulting in a significant decrease in PCARBS in women but not in men (Fig. 2B).

## Discussion

To the best of our knowledge, the present study is the first to determine the sex-specific metabolic changes after the consumption of a honey-based gel in healthy humans via the evaluation of various redox biomarkers and physiological parameters. The results revealed a significant improvement of the antioxidant defense system of the participants, as indicated by the increase in TAC and GSH levels and the decrease of TBARS and PCARBS levels in combination with a decrease of the diastolic and mean BP of the participants, without affecting their hematological profile.

Honey was considered from antiquity as a natural food product with high nutritional importance, used in numerous medical treatments worldwide due to its healing, anti-inflammatory, and antibacterial properties (57). Additionally, evidence in the literature has also widely debated the chemo-preventive, immunoregulatory, antioxidant and antiatherogenic properties of honey (58-61). Nevertheless, information regarding the properties of Greek honey in literature and notably from *in vivo* studies are scarce even though Greece is part of the eastern Mediterranean, creating a rich and diversified flora habitat (62,63). This rich flora biodiversity combined with the overflowing sunshine, creates an ideal environment resulting in a variety of honey types originating from pine, thyme, fir and other conifers (64,65).

According to our *in vitro* results with regard to the honey-based gel, a high antioxidant capacity was observed (TPC, 129 mg GAE/100 g honey; and ABTS assay,  $IC_{50}$ =0.88 mg/ml), compared with other published studies, including raw honey samples. For example, a previous study conducted at the laboratory of the authors investigated the

antioxidant and antibacterial capacity of 21 honey types produced on the highest mountain of the country, Mount Olympus, compared with Manuka honey (66). Regarding the results, the honey-based gel used in the present study, exhibited the lowest  $IC_{50}$  value, determined using an ABTS assay, compared with both the 21 different Greek honey types and Manuka (66). Bazaid *et al* investigated the scavenging activity of Manuka honey using ABTS RSC assay among other assays (67). Concerning the results of the present study, the honey-based gel exhibited a lower  $IC_{50}$  value (0.88 mg/ml) than the assessed Manuka honey (4.49 mg/ml) (67). The present study is part of a larger project investigating Greek honey bioactivity. Specifically, the antioxidant capacity of various types of honey is being investigated both *in vitro* and *in vivo*, as well as their effect on the redox state and physiological profile of the human body. In the present investigation, *in vitro* measurements concerning the honey-based gel were performed in order to establish its polyphenolic content as well as its ability to inhibit the ABTS radical formation. Interestingly, the results of the present study differ from previous data on the bioactivity of honey. For instance, Chau *et al*, after examining various types of honey, such as forest honey, indicated a TPC value ranging from 19.7 to 85 mg GAE/100 g honey (68), which is lower compared to the honey-based gel (129 mg GAE/100 g honey) used in the present study. Concerning a study that assessed 105 different types of raw honey, produced by 3 countries, the honey-based gel used in the present study exhibited higher polyphenolic content from all honey samples except for the buckwheat honey (69). It is plausible that the high level of TPC, determined in the product used in the present study, may be due to substances such as caffeic acid, quercetin and kaempferol which are also found in honey and contribute to its antioxidant activity (70,71). In fact, the honey-based gel used is a mix of 87% fir honey and pollen. Bee pollen is produced by the pollen of flowering plants after mixing with nectar and bee

secretions (72). Pollen, in addition to lipids, sugars, proteins, vitamins, carbohydrates, and amino acids, contains polyphenols such as flavonoids (73) that are related to its antioxidant properties (74). Based on this, it is hypothesized that part of the honey-based gel antioxidant capacity and polyphenolic content is due to the presence of pollen.

In the present study, no significant changes in the hematological profile of the participants were detected, however, in physiological measurements, a significant decrease was observed in diastolic BP and mean arterial BP. Hypertension is known to be a major risk factor for renal and cardiovascular diseases (75). Furthermore, oxidative stress is also involved in the pathogenesis of hypertension, although evidence in the literature indicates the possibility of hypertension leading to oxidative stress development (76,77). Altogether, the beneficial effects of antioxidants in diminishing oxidative damage and attenuating or decreasing high BP, endorse the negative role of oxidative stress in the management of hypertension (78).

A previous study investigated the effects of honey on redox biomarkers evaluated on kidney samples of rats with both diabetes mellitus and hypertension (79). Results from the aforementioned study revealed an increase in left kidney intracellular GSH and reduced/oxidized GSH (GSH/GSSG) ratio after honey supplementation for 3 weeks in rats (79). It is evident that GSH plays a key role in reactive species scavenging and xenobiotic detoxification (80), processes that are closely related to the antioxidant properties of honey (81). The present study revealed a significant increase in GSH levels after the consumption of the honey-based gel. The results from a previous study support the role of honey in the regulation of GSH levels, since diabetic rats that consumed honey for 4 weeks exhibited a significant increase in GSH levels in the kidney tissue (82). In the same study, a significant increase was also found in total antioxidant status levels (82). The aforementioned results are in agreement with the results of the present study, since an increase in the levels of GSH and TAC was observed.

Perturbations in the redox status were also evident by changes in the TBARS and PCARB levels and the findings in the present study are similar with other studies (83-85) where honey consumption resulted in significant decreases of MDA levels, a commonly used marker of oxidative stress that utilizes TBA reagent, for detecting oxidation products of unsaturated fatty acids (84), of female athletes (83) and PCARB following a 0.2 g/kg body weight/day of Tualang honey supplementation for 18 days (85).

One of the aims of the present study was to assess potential differences between men and women on blood redox biomarkers and physiological profile responses following the consumption of a honey-based gel. The results revealed that women had a greater response since there were significant decreases in diastolic BP and mean arterial BP and several blood redox biomarkers. This could be explained by the greater presence of estrogen in the female body (86). Indeed, estrogen levels have a positive correlation with the antioxidant capacity of the plasma and the antioxidant enzymes expressed throughout the menstrual cycle (87) and can prevent lipid peroxidation (88). The antioxidant potential of estrogens, may also have a direct effect on free RSC activity (89), a hypothesis that has been verified through *in vitro* and *ex vivo* animal

studies (90). However, a direct correlation between the antioxidant enzyme system and estrogens has yet to be determined in humans (91).

Limitations of the present study constitute the small sample size and the lack of further biomarkers evaluated in human blood in order to come to safe conclusions regarding the effects on metabolic health. Finally, the evaluation of time-dependent alterations in the levels of the proposed biomarkers during a more extended supplementation period (>14 days) would ensure comparability and reproducibility among future studies that will determine redox and metabolic changes after the consumption of various honey types in different populations. The findings of the present study indicate that the consumption of the natural honey-based gel results in significant changes in BP and indices of the redox status, that are more evident in women. Further studies should assess different dosages and length of time of supplementation.

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### Availability of data and materials

All data generated or analyzed during this study are included in this published article.

### Authors' contributions

DK and AZJ contributed to the conceptualization, supervision, project administration, data analysis and curation of the study, as well as the correction and editing of the manuscript. AP contributed to the methodology, investigation, data analysis and curation, as well as the writing and preparation of the original manuscript. PS contributed to the methodology, investigation, data analysis and curation. MK contributed to the data analysis, writing and preparation of the original manuscript. KP oversaw the methodology, data analysis, writing and preparation of the original manuscript. ZS conducted the formal analysis, wrote the original manuscript, as well as corrected and edited the manuscript. DK and AZJ confirm the authenticity of all the raw data. All authors have read and agreed to the published version of the manuscript.

### Ethics approval and consent to participate

The Institutional Review Board of the Department of Physical Education and Sport Science of the University of Thessaly (protocol ID: 1722/9-12-2020) approved the methods, procedures, and ethics of this study. Procedures agreed with the 1975 Declaration of Helsinki as revised in 2013. All participants provided their written consent for participation after they were

informed about the purpose, methodology and possible risks associated with the study.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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