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Effect of metabolaid® on pre- and stage 1 hypertensive patients: A randomized controlled trial

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ABSTRACT

Many patients with stage 1 hypertension have difficulties to achieve lifestyle changes to avoid the progression of the disease. The use of natural alternatives with demonstrated blood pressure-regulating properties is instrumental at this stage, avoiding the side effects found in some pharmaceutical drugs. In this context, previous studies have indicated the possibility that a botanical nutraceutical product based on the combination of *Lippia citriodora* and *Hibiscus sabdariffa* extracts has hypotensive properties in overweight and obese individuals. Therefore, we aimed to evaluate the antihypertensive properties of this dietary supplement, as well as the effect on anthropometric and circulating parameters in pre-hypertensive and early stage 1 hypertensive patients (n = 84). The nutraceutical product, rich in polyphenolic compounds, has been assessed in a 6-week randomized, double-blind, placebo-controlled trial with pre-hypertensive and early stage 1 hypertensive (non-medicated) individuals. Participants consumed early in the morning in fasting conditions 2 capsules/day containing each one 250 mg of the polyphenolic extracts. Anthropometric and blood parameters as well as punctual and continuous blood pressure monitoring were determined in placebo and experimental groups. As a result and compared to baseline values, volunteers showed a significant reduction of average daily systolic/diastolic blood pressure as well as in daytime diastolic/systolic, nighttime diastolic blood pressure and in % dipper. Intergroup analysis revealed that the consumption of the plant extract resulted in a significant reduction of body fat content (−1.26%; $p < 0.05$) and nocturnal systolic blood pressure (−16.60 mmHg; $p < 0.05$) as well as an improvement of the dipper status (3.18%; $p < 0.01$). In conclusion, these results suggest that the nutraceutical acts as a main regulator of the individual's blood pressure towards healthier values, and therefore may be useful for pre-hypertensive/pre-medicated individuals.

1. Introduction

Cardiovascular diseases are the leading cause of death in industrialized countries and are strongly related to arterial hypertension (HT). Furthermore, the correlation between HT and the risk of cardiovascular disease is highly positive and independent from other risk factors. Otherwise said, the higher the blood pressure (BP), the greater the chances to have a heart attack. In this vein, the development of HT

Abbreviations: **AAMI**, Association for the Advancement of Medical Instrumentation; **AMPK**, AMP-activated protein kinase; **BHS**, British Hypertension Society; **BIA**, bioelectrical impedance analysis; **BMI**, body mass index; **BP**, blood pressure; **CASP**, central aortic systolic pressure; **DP**, diastolic pressure; **ESH**, European Society of Hypertension; **GGT**, gamma glutamyl transferase; **GPT**, glutamic-pyruvic transaminase; **HDL**, high density lipoproteins; **HS**, *Hibiscus sabdariffa*; **HT**, hypertension; **KS**, Kolmogorov-Smirnov; **LC**, *Lippia citriodora*; **LDL**, low density lipoproteins; **MAP**, mean arterial pressure; **NO**, nitric oxide; **SD**, standard deviation; **SP**, systolic pressure; **PP**, pulse pressure.

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depends on the genetic background of the individual, environmental factors and associated pathologies. Surprisingly, the majority of these risk factors, particularly environmental ones, can be prevented or treated (Oliveras & de la Sierra, 2014; Oparil & Schmieder, 2015; Samadian et al., 2016; WHO, 2007).

According to the major health organizations, such as the Task Force for Management of Arterial HT, the European Society of Cardiology and the American College of Cardiology, normal BP values are < 120 mmHg for systolic pressure and < 80 mmHg for diastolic pressure (Whelton et al., 2018). However, when an individual's systolic and diastolic pressure are in the 120–139 mmHg and 80–89 mmHg range, respectively, they are diagnosed with prehypertension. In this context, prehypertension is generally considered as an alert signal for subsequent HT development rather than a pathology by itself. If preventive measures are not applied immediately, these patients could eventually progress towards HT. In this scenario, stage 1 HT corresponds to ranges of 140–159 mmHg for systolic hypertension and 90–99 for diastolic blood pressure. Finally, stage 2 HT is considered when systolic blood pressure is ≥160 mmHg and ≥100 for diastolic blood pressure.

The high prevalence of HT in the adult population around the world is a major public health problem. The number of annual deaths worldwide, as a result of high blood pressure, is increasing (Kearney et al., 2005; Mendis, 2010). In the medical practice, when an individual is diagnosed with prehypertension or early stage 1 HT, it is generally recommended to adopt certain lifestyle habits in order to help to reduce their blood pressure before moving on towards hypotensive medications. These lifestyle interventions include more hours/week of physical activity and consuming a balanced diet. However, BP improvements are not immediate and very often affected patients are discouraged and abandon the lifestyle changes. In this critical moment, the consumption of certain natural dietary supplements with hypotensive properties may help patients to better control their BP.

This is aggravated by the fact that in the majority of cases, prehypertension or early stage 1 HT appears in overweight and obese people. Obesity is not solely an excess of adipose tissue in the organism. The pathology is generally associated with metabolic, oxidative and inflammatory alterations, reaching pandemic proportions in developed countries and increasing the expenses for Health Care Services in the Community. For this reason, metabolic syndrome is a more appropriate terminology, as it comprises increased adipose tissue mass, endothelial dysfunction, dyslipidemia, atherosclerosis, insulin resistance and HT (Furukawa et al., 2004; Luna-Luna et al., 2015).

Taking into account the high prevalence of HT, our research group studied the effect of a nutraceutical product that combines the aqueous polyphenolic extracts of *Lippia citriodora* (LC) and *Hibiscus sabdariffa* (HS). LC originates from South America, but was imported to Europe, where it is widely cultured. It possesses a lemon-like aroma and is used in herbal tea preparations, as it is known for its antispasmodic, antipyretic, sedative and digestive properties (El-Hawary et al., 2012). HS has been used traditionally in herbal drinks, in hot and cold beverages, as a flavouring agent in the food industry and as a botanical medicine (Da-Costa-Rocha et al., 2014). In our previous reports, we have observed that the resulting herbal combination (LC + HS) is capable of activating AMP-activated protein kinase (AMPK) *in vitro* and reduce intracellular lipid accumulation in cultured adipocytes through the regulation of different metabolic pathways (Herranz-López et al., 2019). The effects observed with the extract have also been corroborated in hyperlipidemic animal models (Herranz-Lopez et al., 2015; Joven et al., 2012; Lee et al., 2018). Furthermore, the combination of LC + HS has been reported to reduce body fat and improve the general health status of overweight subjects compared to the placebo group (Boix-Castejon et al., 2018; Herranz-López et al., 2019). All these effects prompted us to explore its potential use in the relief of pathologies related to obesity, including HT.

To this end, the main objective of this work was to evaluate the antihypertensive properties of the herbal combination (LC + HS), a nutraceutical product named MetabolAid®. As a second objective, data were collected in order to study the ability of this polyphenolic combination to modulate certain anthropometric parameters, as well as several circulating values in pre-hypertensive and early stage 1, non-medicated hypertensive patients participating in the study.

2. Materials and methods

2.1. Supplement formulation

MetabolAid® (patent application number P201731147) capsules were kindly provided from Monteloeder SL (Elche, Spain). The capsules contained 500 mg of a polyphenolic mixture isolated from HS calyxes and LC leaves whose composition has been previously determined by HPLC coupled to mass spectrometry (Herranz-López et al., 2019). Briefly, the supplement was composed of a combination at a weight ratio (w/w) of 65:35 of LC polyphenolic extract (25% phenylpropanoids, dry weight) and HS polyphenolic extract (10% anthocyanins, dry weight), as declared by the manufacturer. The major anthocyanins identified were delphinidin-3-O-sambubioside and cyanidin-3-O-sambubioside, and the main phenylpropanoids were verbascoside and isoverbascoside (see Fig. 1-Supplementary for detailed composition).

2.2. Participants

563 pre-hypertensive and stage 1 hypertensive patients were selected from the Raval Health Center located in the city of Elche (Spain). 479 patients were excluded because they did not meet the inclusion criteria, mainly because they were under medication. Finally, 84 patients were included in the study according to the following inclusion criteria:

a) Adults pre-hypertensive /stage 1 HT with no pharmacological treatment;
b) Participants accept to undergo hygienic-dietetic intervention;
c) Participants were willing to ingest the nutritional supplement containing the polyphenolic extract;
d) Participants signed the informed consent.

The following exclusion criteria were used:

a) Individuals under 18 years of age;
b) Individuals with BMI > 35;
c) Patients with high cardiovascular risk (stage 2 HT);
d) Patients with previous chronic diseases;
e) Presence of allergy to the compounds of the product and placebo according to the technical data sheet.

Participants were recruited by the ALUMBRA sanitary information analysis system service. To this end, the lists of patients with a history of HT were provided by the medical staff and those who met the established inclusion criteria were selected.

Before participating in the study, the researchers informed the subjects about the product and the study procedures. All subjects provided written informed consent before participating. The study was approved by the Ethics Committee of Miguel Hernández University (Elche, Spain) (reference IBM.VMM.01.17) and was conducted according to the Helsinki Declaration criteria (1983 version). The study was registered in Clinical Trials with the reference number NCT03507023.

2.3. Trial design

The study was a randomized, double-blind, placebo-controlled trial lasting 6 weeks based on previous studies from our group (Boix-Castejon et al., 2018; Herranz-López et al., 2019). After recruitment, the volunteers were randomized into the placebo/L1 (n = 41) or experimental/L2 group (n = 43) (Fig. 1). Both groups presented non-significant differences regarding BP parameters at the beginning of the study. The

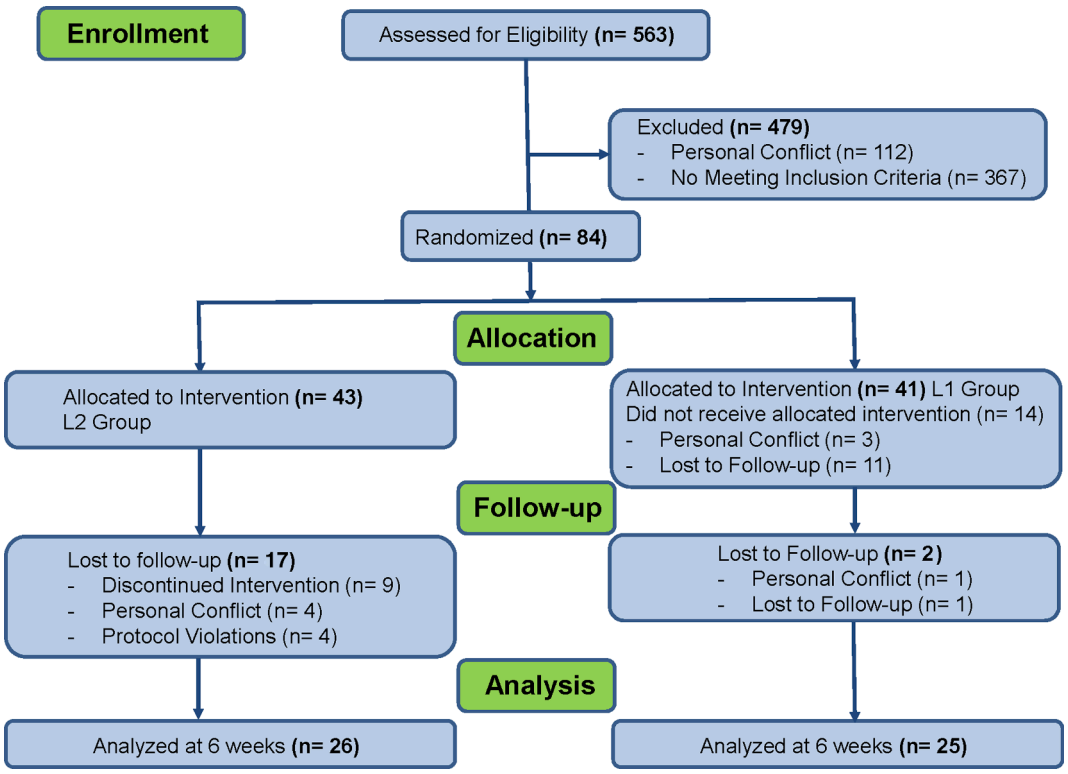

Fig. 1. Participant flow diagram. L1 corresponds to the placebo group and L2 to the dietary supplement group.

placebo group received two daily capsules of 370 mg each of crystalline microcellulose per day. The experimental group received two capsules containing each one 250 mg of Metabolaid® + 120 mg of crystalline microcellulose. Therefore, the daily consumption was 500 mg of Metabolaid®.

The MetabolAid® and placebo capsules were prepared so that they were the same size, smell and color. The volunteers were instructed to take two capsules 20–30 min before breakfast every day for 6 weeks. In addition, they received indications to change certain dietetic habits (reduction of salt, saturated fat and sugar in foods) and to walk daily for at least 30 min. No personal diet nor specific exercise programs were provided.

The compliance of the subjects with the intake of capsules and the use of the worn ambulatory device was evaluated at each visit to the Raval Health Center (Elche, Spain), or through telephone interviews every week. At the end of the study, a total of 16 and 17 participants were excluded from the placebo and experimental group, respectively (Fig. 1).

2.4. Intervention

2.4.1. Anthropometric measurements and blood analysis

Anthropometric measures were taken at the beginning of the study, week 2 and week 6 of the intervention period, including body weight and height. Percentage of fat, lean mass and body water were determined using a bioelectrical impedance analysis (BIA) device (Tanita BC-545 N, Tanita Corporation, Japan). The body mass index (BMI) was derived from body weight and height using the equation BMI = Body weight (Kg) / height^2 (m^2).

Blood analyses were performed in the same health center. Blood samples were extracted in fasting conditions, at the beginning and end of the study.

2.4.2. Blood pressure determinations

The determination of BP in the health center was carried out by trained personnel following the standards proposed by the European Society of Hypertension (Williams et al., 2018). Systolic and diastolic blood pressures at rest and heart rate were recorded at the beginning of the study, week 1, week 2, week 4 and week 6 of the intervention using a BP monitor MC3100 + validated voltage monitor (HealthStats, Singapore).

Also, a BPro® device (HealthStats, Singapore) was provided to each patient for one day at different moments of the intervention as a continuous blood pressure monitor. BPro® is validated by the BHS (British Hypertension Society), by the AAMI (Association for the Advancement of Medical Instrumentation) and by the ESH (European Society of Hypertension) (MedTach, 2019). The associated software of the device allowed to have an accurate macroscopic view of BP patterns during 24 h.

Parameters provided by the BPro® include the hourly systolic and diastolic BP measurements, from which the 24 h mean and daytime/nighttime average measurements were calculated. The percentage of Dipper/Non-Dipper was also evaluated by the BPro®. All these determinations were performed at the beginning of the study, and after 1, 2, 4 and 6 weeks of product consumption.

BP follows a circadian pattern, with higher blood pressure values during the day and lower at night (dipper pattern). On the contrary, in individuals that are non-dippers, such as those with HT, no significant decrease in night BP was observed (Islam, 2017). However, an extreme decrease of BP at night has been recently associated with other cardiovascular diseases such as cerebral ictus or vascular diabetic complications, although evidences remain weak at present (Islam, 2017; Williams et al., 2018). The % Dipper was calculated according the equation: (Average Day Systolic BP - Average Night Systolic BP) × 100% / Average Day Systolic BP). The obtained values are associated to a different prognosis. These include: ≤0% (reverse dipper); 0–10% (non-dipper); 10–15% (normal dipper) and >15% (extreme dipper). The profiles with worse prognosis are associated with the values of the reverse dipper (≤0%) and extreme dipper (>15%). Normal dipper (10–15%) is considered the healthiest pattern.

Other parameters provided by the BPro® device are: central aortic systolic pressure (CASP), pulse pressure (PP) and mean arterial pressure

(MAP). For CASP, the BPro® determines the average of the progressive motion of the pulse wave in a point by considering the heart rate of the point divided by 4. Therefore, CASP allows comparing the intracardiac pressure respect to peripheric (brachial) pressure. CASP range = 101–136 mmHg, and CASP > 117 mmHg indicates risk of angiopathy. PP indicates the flexibility of vessel walls. PP is determined as the difference between systolic pressure (SP) and diastolic pressure (DP): PP = SP-DP. PP > 50 mmHg indicates risk of atherosclerosis. Finally, MAP indicates the mean perfusion pressure into the different organs. MAP > 60 mmHg indicates a good perfusion pattern with a low risk of ischemia. On the other hand, MAP < 60 mmHg indicates an increased risk of ischemia in peripheral organs.

2.5. Statistical analysis

The statistical analysis was carried out using the Graphpad Prism software. The results were expressed as means ± standard deviation (SD). The anthropometric, biochemical and vital values (intragroup) were analyzed by the unpaired Student *t* test. On the other hand, the vital signs and the anthropometric parameters (intergroup) were analyzed by means of the paired Student *t* test. The values obtained through the BPro® devices were analyzed by one-way ANOVA. The outcome variables were evaluated to determine their compliance with the normal distribution and transformed if necessary by a Shapiro-Wilk test. The p values reported were bilateral and values of 0.05 or less (0.01, 0.001 and 0.0001) were considered statistically significant for the comparisons with the baseline (start of the study) and between the groups (placebo *vs* supplement) at the same moment of the study.

3. Results

3.1. Anthropometric parameters

The anthropometric parameters in the intragroup analysis, obtained by comparing the baseline data at the beginning of the intervention study versus the follow-up at 3 weeks, did not show significant differences in placebo group. On the other hand, the results obtained showed a significant decrease in the percentage of fat (approximately 1%), in patients of the supplemented group after six weeks of intervention (Table 1). Regarding weight, there was a significant reduction of approximately 1 kg after six weeks in the supplemented group (Table 1). In the inter-group analysis, significant differences were observed when comparing the values obtained in the placebo group versus the supplemented group for fat percentage at the end of intervention (Table 2).

Table 1
Evolution in anthropometric parameters comparing values at the beginning (t = 0) *vs* the end of the 2nd week and 6th week into the same group (placebo or supplemented group) (intragroup analysis). Significance was established at: **p < 0.01. Means ± SD.

	Placebo (n = 25)			Supplemented (n = 26)		
Anthropometric parameters	**t = 0**	**2nd week**	**6th week**	**t = 0**	**2nd week**	**6th week**
Weight (Kg)	78.85 ± 14.48	78.96 ± 14.49	78.78 ± 14.71	88.91 ± 15.46	88.92 ± 15.49	87.93 ± 16.18 **
Fat mass (%)	33.72 ± 8.40	33.85 ± 8.51	33.11 ± 8.54	35.15 ± 9.30	34.49 ± 9.69	33.89 ± 9.65 **
Body water (%)	48.32 ± 6.84	48.44 ± 7.21	48.40 ± 7.05	47.16 ± 5.94	47.67 ± 6.19	47.60 ± 6.37
Muscle mass (%)	51.03 ± 11.79	50.67 ± 12.31	51.44 ± 10.90	53.88 ± 8.91	54.20 ± 9.01	54.16 ± 9.03
BMI (kg/m^2)	28.58 ± 4.62	28.62 ± 4.60	28.55 ± 4.56	31.37 ± 6.35	31.38 ± 6.40	31.17 ± 6.43

Abbreviations: Body mass index (BMI), t = 0 (baseline).

3.2. Circulating parameters

Table 3 shows changes in circulating parameters at the end of intervention in the same group. The placebo group only presented significant decreases in creatinine and HDL (high density lipoproteins). Meanwhile, the supplemented group presented significant decreases in creatinine, LDL (low density lipoproteins), GPT (glutamic-pyruvic transaminase) or GGT (gamma glutamyl transferase) and red cell number. However, no significant changes were observed when comparing the evolution (6 weeks respect to beginning of intervention) of the circulating parameters between both groups (placebo *vs* supplemented) (Table 1-Supplementary).

3.3. Parameters related to blood pressure

3.3.1. Continuous monitoring by BPro® ambulatory devices

Table 4 compares the different values obtained from the continuous monitoring of the BPro® devices between patients in the placebo group and the supplemented group. Table 5 shows the intergroup comparison. The weekly results indicated a generalized significant improvement in BP in the supplemented group (Table 4). The improvements were observed in the weekly evolution of total systolic and diastolic pressure as well as in daytime diastolic, daytime systolic, nighttime diastolic blood pressures and in % dipper. Interestingly, improvements began to be evident from the second week of consumption of the supplement for total systolic BP and systolic BP at day and at night (Figs. 2 and 3-Supplementary).

The analysis of the % dipper in the placebo group showed a tendency from the beginning to maintain a non-dipper pattern (0–10%). On the other hand, in the treated group, an upward significant trend towards the normal dipper pattern was observed (Table 5) (Fig. 4-Supplementary). Regarding CASP, the BPro® values decreased in the group consuming the supplement towards the non-risk range. However, no significant CASP changes were noticed in the placebo group (Table 4). In addition, PP values improved in the supplemented group compared with the placebo, where significant changes were not noticed (Table 4). Finally, MAP values indicated that both groups displayed an optimal perfusion in peripheral organs (Table 4). Altogether, total systolic pressure, nighttime systolic pressure, % dipper, CASP and MAP in the supplemented group presented significant improvements compared to placebo (Table 5).

3.3.2. Digital pressure monitor

The data obtained with the BPro® device were corroborated with the data from the sphygmomanometer (Table 6). A significant improvement of both systolic and diastolic blood pressures was observed in the

Table 2
Differences in anthropometric parameters comparing changes during the intervention between both groups (placebo *vs* supplemented) (intergroup analysis). Significance was established at: *p < 0.05. Means ± SD.

	Differences after two weeks (Value at 2 weeks – Value at t = 0)		Differences after six weeks (Value at 6 weeks – Value at t = 0)	
Anthropometric parameters	**Placebo**	**Supplemented**	**Placebo**	**Supplemented**
Weight (Kg)	0.11 ± 0.15	0.02 ± 0.14	−0.07 ± 0.25	−0.98 ± 0.33 *
Fat mass (%)	0.14 ± 0.18	−0.66 ± 0.43	−0.60 ± 0.53	−1.26 ± 0.41 *
Body water (%)	0.12 ± 0.21	0.51 ± 0.38	0.08 ± 0.22	0.44 ± 0.42
Muscle mass (%)	0.36 ± 0.25	0.32 ± 0.52	0.40 ± 0.54	0.28 ± 0.54
BMI (kg/m^2)	0.04 ± 0.06	0.01 ± 0.05	−0.03 ± 0.10	−0.20 ± 0.19

Abbreviations: Body mass index (BMI), t = 0 (baseline)

Table 3
Evolution of circulating parameters comparing beginning *vs* the end of intervention into the same group (placebo or supplemented group) (intragroup analysis). The significance was established at: *p < 0.05, **p < 0.01. Means ± SD.

	Placebo (n = 25)		Supplemented (n = 26)	
Blood parameters	t = 0	6th week	t = 0	6th week
Glucose (mg/dl)	106.3 ± 29.32	108.4 ± 24.04	102.1 ± 18.28	101.6 ± 18.29
Creatinine	0.87 ± 0.17	0.82 ± 0.17 **	0.84 ± 0.16	0.80 ± 0.17 *
Uric acid (mg/dl)	5.19 ± 1.34	5.31 ± 1.29	5.17 ± 1.24	5.27 ± 1.29
Triglycerides (mg/dl)	159.5 ± 114.6	164.2 ± 110.7	127.8 ± 66.48	127.5 ± 67.53
Total cholesterol (mg/dl)	220.0 ± 36.02	216.2 ± 37.69	209.9 ± 40.10	206.2 ± 41.61
HDL (mg/dl)	57.54 ± 16.26	54.24 ± 13.83*	55.85 ± 15.67	56.25 ± 16.66
LDL (mg/dl)	135.2 ± 35.16	133.2 ± 34.02	131.3 ± 35.22	123.2 ± 33.24 *
GOT/AST (U/L)	36.50 ± 63.09	38.15 ± 62.90	23.50 ± 7.33	22.58 ± 6.06
GPT/ALT (U/L)	32.30 ± 12.64	34.90 ± 24.52	30.21 ± 13.94	26.92 ± 11.30 *
GGT (U/L)	39.05 ± 26.11	39.95 ± 26.80	27.83 ± 18.46	26.17 ± 17.17*
Erythrocyte (x 10^6 μL)	4.92 ± 0.49	4.94 ± 0.44	4.89 ± 0.42	4.83 ± 0.43 *
Haemoglobin (g/dL)	14.69 ± 1.43	14.90 ± 1.35	14.80 ± 1.44	14.63 ± 1.41
Haematocrit (%)	44.63 ± 4.53	44.73 ± 4.11	44.50 ± 4.09	43.81 ± 4.23
MCV (fL)	87.80 ± 13.69	83.35 ± 22.80	91.15 ± 4.43	90.68 ± 3.97

Abbreviations: Gamma-glutamil transferase (GGT), glutamic-oxaloacetic transaminase/aspartate transaminase (GOT/AST), glutamic-pyruvic transaminase/alanine transaminase (GPT/ALT), high density lipoproteins (HDL), low density lipoproteins (LDL), mean corpuscular volume (MCV), t = 0 (baseline).

supplemented group starting from the second week taking the supplement. The results indicated a progressive improvement until the last measurement registered in consultation (sixth week). The most significant improvement was observed for the supplemented group compared with the placebo at the sixth week in systolic pressure (Table 7).

4. Discussion

Arterial HT is the risk factor with the greatest impact on cardiac and cerebrovascular mortality in both women and men (Messerli et al., 2007). BP control requires complex integration of regulatory mechanisms across multiple physiological systems. A sustained increase in arterial pressure therefore reflects a failure of one or more of these controls (Bolívar, 2013). This research focuses on the study of the effects of a polyphenolic dietary supplement (LC + HS) in HT, as a major component of metabolic syndrome (Savica et al., 2010), providing a new research framework.

Dietary control in hypertensive patients is very important to prevent, improve or eradicate mild HT. However, adherence to dietary guidelines is generally not maintained in the long term (Vairavamurthy et al., 2017). Previous studies have demonstrated the hypotensive effect of Metabolaid® (Boix-Castejon et al., 2018; Herranz-López et al., 2019). The results suggest that this combination of LC + HS acts as a main regulator of the individual's blood pressure. Indeed, arterial pressure showed a significant improvement in all parameters comparing baseline to week 6 (systolic, diastolic, day and night tension) only in the supplemented group. Comparing both groups, systolic night pressure and % dipper showed significant differences. This suggests a hypotensive effect that starts to be evident at night, when individuals are resting (compare % dipper *vs* non-dipper pattern). However, this improvement is not totally established during the day when individuals are more active. Altogether, this suggests a tendency of BP towards a normal circadian rhythm as a first step to correct HT. In addition, PP and CASP show a significant improvement towards a lower risk to develop angiopathy compared to placebo. The changes in HT were accompanied by a significant decrease in LDL, a circulating parameter that depends on diet and exercise. In this context, dyslipidemia and HT are pathological conditions that favour endothelium damage, particularly in the case of oxidized LDL (Hurtubise et al., 2016). Since reaching normal BP is a long process, these results suggest the necessity to reinforce the hypotensive/hypolipidemic effects of Metabolaid® by introducing a customized program of exercise and balanced diet that has not been implemented in the present study.

In this vein, we have observed that the supplemenís effects are enhanced when it is administered in the context of diet + exercise (Boix-Castejon et al., 2018; Herranz-López et al., 2019). This is supported by the observation that anthropometric parameters (weight, fat % and BMI) improved in previous reports from our laboratory (Boix-

Table 4
Evolution in BPro® measurements comparing values at the beginning (t = 0) *vs* the end of the 1st week, 2nd week, 4th week and 6th week into the same group (placebo or supplemented group) (intragroup analysis). Significance was established at: *p < 0.05, **p < 0.01, ***p < 0.001. Means ± SD.

	Placebo (n = 25)					Supplemented (n = 26)				
	t = 0	1st week	2nd week	4th week	6th week	t = 0	1st week	2nd week	4th week	6th week
Total Systolic (mmHg)	138.7 ± 15.63	133.2 ± 30.64	137.2 ± 20.80	139.3 ± 20.84	136.7 ± 20.77	149.5 ± 27.78	140.7 ± 17.62	135.7 ± 18.56**	136.5 ± 11.26*	130.2 ± 18.56***
Systolic day (mmHg)	143.9 ± 15.24	141.7 ± 13.98	142.5 ± 21.58	143.7 ± 22.53	140.5 ± 21.26	147.1 ± 18.27	144.6 ± 18.00	139.6 ± 16.95**	142.4 ± 14.94	136.1 ± 18.93**
Systolic night (mmHg)	131.3 ± 17.23	135.0 ± 17.09	128.9 ± 17.36	130.7 ± 19.01	133.5 ± 22.97	139.1 ± 28.53	131.8 ± 18.20	126.6 ± 17.73**	130.9 ± 13.03	122.5 ± 19.40**
% dipper	9.15 ± 5.65	4.50 ± 8.34*	8.54 ± 5.22	8.44 ± 3.75	7.30 ± 5.79	6.94 ± 6.02	8.46 ± 5.13	8.80 ± 5.00	8.45 ± 4.63	10.13 ± 4.84*
Total diastolic (mmHg)	89.76 ± 11.78	90.76 ± 10.86	86.08 ± 11.90	89.40 ± 14.82	86.36 ± 13.86	93.56 ± 13.13	91.56 ± 11.98	87.16 ± 12.45*	89.88 ± 13.89	84.36 ± 14.76**
Diastolic day (mmHg)	93.88 ± 12.34	92.85 ± 11.04	90.85 ± 13.45	93.04 ± 15.63	89.50 ± 14.78	96.26 ± 13.43	95.48 ± 12.65	90.57 ± 12.61	93.35 ± 15.84	88.26 ± 15.26*
Diastolic night (mmHg)	84.72 ± 12.34	87.36 ± 10.07	81.16 ± 10.96	83.92 ± 13.89	81.92 ± 12.75	87.60 ± 14.30	86.12 ± 11.52	81.60 ± 12.11*	84.52 ± 13.32	78.88 ± 14.79**
CASP (mmHg)	145.3 ± 23.54	142.6 ± 23.69	143.7 ± 20.52	142.2 ± 21.90	140.4 ± 21.65	149.5 ± 29.89	138.3 ± 21.40*	135.4 ± 17.04**	135.5 ± 14.21**	134.3 ± 22.07***
PP (mmHg)	59.64 ± 19.92	54.20 ± 20.56	56.40 ± 19.95	52.00 ± 14.04	56.92 ± 15.51	62.52 ± 22.89	54.52 ± 13.10	54.16 ± 12.98*	49.00 ± 15.41**	53.40 ± 17.59*
MAP (mmHg)	115.3 ± 1360	114.7 ± 14.44	112.2 ± 14.78	111.7 ± 16.19	110.6 ± 14.60*	119.1 ± 17.63	111.6 ± 12.60**	109.3 ± 13.23***	109.4 ± 12.14**	106.7 ± 16.39***

Table 5
Differences in BPro® measurements (#week-(t = 0)) comparing changes during the intervention between both groups (placebo *vs* supplemented) (intergroup analysis). Significance was established at: *p < 0.05, **p < 0.01, ***p < 0.001. Means ± SD.

	1st week – (t = 0)		2nd week – (t = 0)		4th week – (t = 0)		6th week – (t = 0)	
	Placebo	**Supplemented**	**Placebo**	**Supplemented**	**Placebo**	**Supplemented**	**Placebo**	**Supplemented**
Total Systolic (mmHg)	−5.50 ± 6.41	−8.78 ± 5.05	−1.53 ± 3.38	−13.74 ± 4.44*	0.54 ± 3.42	−12.96 ± 5.68	−2.04 ± 3.31	−19.30 ± 4.64**
Systolic day (mmHg)	−2.15 ± 2.72	−2.56 ± 4.18	−1.42 ± 3.33	−7.56 ± 2.38	−0.15 ± 3.77	−4.68 ± 4.51	−3.42 ± 3.36	−11.00 ± 3.32
Systolic night (mm Hg)	2.11 ± 3.54	−7.24 ± 4.26	−2.38 ± 3.28	−12.44 ± 4.27	−0.58 ± 3.24	−8.20 ± 5.12	−10.15 ± 11.33	−16.60 ± 4.64*
% dipper	−4.65 ± 1.92	1.51 ± 0.93**	−0.61 ± 1.25	1.86 ± 1.12	−0.72 ± 1.36	1.51 ± 1.23	−1.85 ± 1.20	3.18 ± 1.34**
Total diastolic (mmHg)	1.00 ± 2.35	−2.00 ± 2.57	−3.68 ± 1.96	−6.40 ± 2.64	−0.36 ± 2.68	−3.68 ± 3.30	−3.04 ± 2.45	−9.20 ± 2.90
Diastolic day (mmHg)	−1.04 ± 2.25	−0.72 ± 2.55	−3.04 ± 1.99	−5.70 ± 2.79	−0.85 ± 2.80	−2.91 ± 3.94	−4.39 ± 2.45	−8.00 ± 3.14
Diastolic night (mmHg)	2.64 ± 2.36	−1.48 ± 2.23	−4.11 ± 1.94	−6.00 ± 2.47	−4.65 ± 4.65	−3.08 ± 3.19	−2.80 ± 2.52	−8.72 ± 3.09
CASP (mmHg)	2.74 ± 3.16	−11.21 ± 5.02	−1.60 ± 3.64	−14.13 ± 4.72*	−3.13 ± 3.21	−14.08 ± 4.80	−4.87 ± 2.95	−15.29 ± 3.79*
PP (mmHg)	−5.44 ± 3.32	8.00 ± 3.91	−3.24 ± 3.04	−8.36 ± 3.64	−7.64 ± 4.06	−13.52 ± 4.83	−2.72 ± 2.79	−9.12 ± 3.99
MAP (mmHg)	−0.60 ± 1.67	−12.44 ± 2.02***	−3.16 ± 2.06	−9.72 ± 2.70	−3.60 ± 2.21	−9.84 ± 2.05*	−4.68 ± 1.97	−12.44 ± 2.02**

Table 6
Evolution in vital signs parameters using the digital pressure monitor and comparing values at the beginning (t = 0) *vs* the end of the 1 st week, 2nd week, 4th week and 6th week into the same group (placebo or supplemented group) (intragroup analysis). Significance was established at: *p < 0.05, **p < 0.01, ***p < 0.001, ***p < 0.0001. Means ± SD.

	Vital signs	t = 0	1 st week	2nd week	4th week	6th week
Placebo	**Heart rate (bpm)**	78.80 ± 15.03	78.88 ± 12.22	82.36 ± 17.65	79.76 ± 11.72	78.44 ± 12.56
n = 25	**Systolic pressure (mmHg)**	154.7 ± 21.86	152.6 ± 24.15	149.5 ± 23.30	149.3 ± 23.03*	146.9 ± 21.40*
	Diastolic pressure (mmHg)	95.36 ± 11.28	95.58 ± 11.67	92.76 ± 14.09*	93.36 ± 15.11	91.00 ± 13.22*
Supplemented	**Heart rate (bpm)**	84.76 ± 15.86	85.72 ± 17.54	86.84 ± 16.57	85.08 ± 15.26	81.08 ± 15.07
n = 26	**Systolic pressure (mmHg)**	160.8 ± 31.11	148.0 ± 18.32**	145.3 ± 18.47***	143.8 ± 13.28**	142.3 ± 22.76***
	Diastolic pressure (mmHg)	97.88 ± 12.42	93.56 ± 11.04**	91.12 ± 12.13***	92.48 ± 13.30*	88.88 ± 15.58***

Table 7
Differences respect to baseline (beginning of the intervention) of vital signs expressed as means ± Std. Error. Intergroup statistical analysis was performed at 2nd and 6th week compared to the baseline (t = 0). Significance was established at: *p < 0.05.

	Differences after two weeks (Value at 2 weeks – Value at t = 0)		Differences after six weeks (Value at 6 weeks – Value at t = 0)	
Vital signs	**Placebo**	**Supplemented**	**Placebo**	**Supplemented**
Heart rate (bpm)	3.56 ± 4.61	2.08 ± 2.94	0.36 ± 2.35	−3.68 ± 2.91
Systolic pressure (mmHg)	−5.24 ± 3.06	−15.48 ± 3.98	−7.84 ± 2.97	−18.48 ± 3.86*
Diastolic pressure (mmHg)	−2.60 ± 1.87	−6.76 ± 1.55	−4.46 ± 1.80	−9.00 ± 1.98

Castejon et al., 2018; Herranz-López et al., 2019), meanwhile only fat % decreased significantly but modestly in the present study. Therefore, diet and exercise management are instrumental for weight reduction in overweight patients. It must be mentioned that participants in the present study were selected according to the HT value at the beginning of the study as the main selection criterion, but not according to their BMI. In this respect, the number of obese (IBM > 30) was balanced in both groups (n = 15 in the placebo and n = 12 in the supplemented group). In addition, participants followed only general indications from the health center, with no personal diet nor physical activity program included. For this reason, improvements in anthropometric parameters were modest or not significant. The indications provided were very general and subjected to the interpretation made by participants. This observation must alert health authorities and consider that more direct interventions need to be implemented to change unhealthy lifestyle habits in affected populations. In the same context, the tendency of circulating lipid parameters showed a modest but positive evolution towards values into the healthy range. Nevertheless, under these unfavourable conditions, the plant extract was capable of improving HT values in the supplemented group.

There are several hypothesis as to the molecular mechanisms behind the observed effects (Joven et al., 2014a, 2014b). In this context, AMPK is a major therapeutic target of the plant extract by controlling lipid accumulation in liver, adipose tissue and bloodstream (Herranz-Lopez et al., 2017). However, the evidences accumulated *in vitro* do not discard other molecular targets such as oxidative stress inhibition and the blockade of inflammatory cytokine secretion (Herranz-Lopez et al., 2012). It is well established that AMPK activation in the majority of tissues promotes activation of fatty acid oxidation and glucose uptake, decreasing at the same time fatty acid and cholesterol synthesis (Ford et al., 2012; Greig et al., 2015; López et al., 2016; Sun et al., 2015; Yeh et al., 2018). These observations support the decrease in transaminases detected in the present study in the supplemented group, suggesting a hepato-protective effect.

Previous studies have suggested that LC and HS extracts may exert a blood-pressure lowering effect, and their mechanisms of action have been elucidated to a certain extent. In the case of LC, though it is not a well-known effect, the active compounds of this plant, especially verbascoside, have been shown to have an effect in lowering blood pressure in animal models (Ragone et al., 2010). Specifically, verbascoside has been shown to reduce BP in rats at 2 h after administration. Similar studies reported a nitric oxide (NO)-mediating relaxing effect in the aorta in rats (Wong et al., 2001). Both direct administration (injection) and oral intake have been studied, with similar results. In fact, the authors report that compared to captopril, which is a drug to treat HT, verbascoside at 10 mg/kg body weight had similar effects to captopril at 5 mg/kg in lowering systolic blood pressure and was better than captopril in lowering diastolic blood pressure.

In the above mentioned study using LC extract (Ragone et al., 2010), blood pressure was lowered using increasing concentrations of an aqueous solution of LC. Based on the tests performed, the hypotensive effect was not due to muscarinic receptors nor NO release. Rather, components of the extract seem to have a direct effect on the smooth muscles, causing a vasorelaxing effect (more technically, a non-competitive contractile blockade). Also, in the heart, a negative cardiac ionotropism (i.e., a weakening of the force used to contract the heart, similar to beta-blocker medication) was detected, which could

contribute to the hypotension. The authors suggest that verbascoside was the major compound involved in the observed effect.

On the other hand, HS extracts have been shown to have several effects on blood vessel endothelial cells to induce blood pressure. These mechanisms include:

- Vasorelaxation effect (Zheoat et al., 2019): Hibiscus acid has a vasorelaxation effect in the aorta, due to the inhibition of Ca^{2+} influx via voltage-dependent Ca^{2+} channels. A similar effect has been observed with garcinia acid of *Garcinia cambogia*, which is an isomer of hibiscus acid.
- Increased NO production (Joven et al., 2014a, 2014b): HS polyphenols increase endothelial NO synthase (eNOS) production, with in turn increases NO. This is similar to other botanical extracts that reduce blood pressure, such as garlic and citric extracts (i.e. vitamin C).
- ACEi (Angiotensin Converting Enzyme Inhibitors) act on the angiotensin-renin-aldosterone system by inhibiting the enzyme that converts angiotensin I into angiotensin II, thus causing an increase in systemic vasodilation of peripheral blood vessels, resulting in a decrease in blood pressure. HS extracts have been shown to stimulate this pathway (Herman, 2020; Ojeda et al., 2010; Salem et al., 2020).
- Decreased blood viscosity through cyclooxygenase inhibitory activity (Christian et al., 2006).

Obviously, the compounds responsible for such activity must be the metabolites derived from the metabolic transformation of the LC and HS combination upon ingestion. Based on our own evidences obtained in cell and animal models and from others, we postulate that the putative candidate compounds that exert the hypotensive effect must be verbascoside, quercetin glucuronide and/or quercetin. In this context, 16% of the total dry weight of the dietary supplement were phenylpropanoids and verbascoside was the major compound in this family, meaning that the daily consumption of phenylpropanoids in the supplemented group was approximately 80 mg (Fernandez-Arroyo et al., 2012; Herranz-Lopez et al., 2015, 2017, Herranz-López et al., 2019, 2020; Joven et al., 2014a, 2014b). However, the identification of the active metabolites in human blood plasma and how these compounds reach their cell/tissue targets related to such effects should be further investigated. Therefore, these studies suggest that the combination of these extracts exerts a hypotensive effect through multifaceted mechanism, as reported (Medina-Remón et al., 2015). In conclusion, the LC + HS nutraceutical presented in this report offers new dietary possibilities in the treatment of pathologies associated to HT which molecular mechanisms need to be assessed in future research.

Ethics statement

All subjects provided written informed consent before participating. The protocol was in accordance with national legal requirements and the Helsinki Declaration for research on human beings and approved by the Ethics Committee of Miguel Hernández University (Elche, Spain) (reference IBM.VMM.01.17). The study was registered in Clinical Trials with the reference number NCT03507023.

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CRediT authorship contribution statement

Marina Boix-Castejón: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **María Herranz-López:** Conceptualization, Methodology, Project administration, Supervision, Validation, Writing - review & editing. **Mariló Olivares-Vicente:** Investigation, Methodology, Software. **Paula Campoy:** Data curation, Methodology. **Nuria Caturla:** Conceptualization, Funding acquisition, Visualization. **Jonathan Jones:** Conceptualization, Funding acquisition, Visualization. **Juan M. Zazo:** Funding acquisition, Investigation, Supervision. **Enrique Roche:** Conceptualization, Investigation, Validation, Visualization, Writing - review & editing. **Vicente Micol:** Conceptualization, Funding acquisition, Project administration, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104583.

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