European Review for Medical and Pharmacological Sciences 2023; 27: 6718-6727

Berberine phospholipid exerts a positive effect on the glycemic profile of overweight subjects with impaired fasting blood glucose (IFG): a randomized double-blind placebo-controlled clinical trial

M. RONDANELLI[1,2], C. GASPARRI[3], G. PETRANGOLINI[4], P. ALLEGRINI[4], D. AVENOSO[4], T. FAZIA[5], L. BERNARDINELLI[5], G. PERONI[3], Z. PATELLI[3], F. MANSUETO[3], A. TARTARA[3], A. CAVIONI[3], A. RIVA[4]

[1]Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Pavia, Italy
[2]IRCCS Mondino Foundation, Pavia, Italy
[3]Endocrinology and Nutrition Unit, Azienda di Servizi alla Persona "Istituto Santa Margherita", University of Pavia, Pavia, Italy
[4]R&D Department, Indena SpA, Milan, Italy
[5]Department of Brain and Behavioral Science, University of Pavia, Pavia, Italy

Abstract. – OBJECTIVE: Berberine is a plant alkaloid known to exert positive metabolic effects. Human studies have confirmed its ability to improve the lipid and glycemic profile. This study aimed to evaluate the potential benefit of oral supplementation of Berberine Phytosome™ (2 tablets/day, 550 mg/tablet) on the metabolic profile of subjects with impaired fasting blood glucose (IFG).

PATIENTS AND METHODS: A total of 49 overweight subjects, 28 females and 21 males, were randomly assigned to either the supplemented group (n=24) or placebo (n=25). We considered glycemia as the primary endpoint and total cholesterol, high-density lipoprotein (HDL), total cholesterol/HLD, low-density lipoprotein (LDL), LDL/HDL, triglycerides, insulin, glycated hemoglobin, Homeostasis Model Assessment (HOMA), ApoA, ApoB, ApoB/ApoA, androgen suppression treatment (AST), alternative lengthening of telomeres (ALT), gamma-glutamyl transferase (GGT), creatinine, and body composition by Dual-energy X-ray Absorptiometry (DXA) as secondary endpoints. These parameters have been assessed at baseline, after 30 days, and after 60 days.

RESULTS: After two months of treatment, through the use of linear mixed effect models, a statistically significant difference between supplemented and placebo groups was observed for glycemia [β=-0.2495% C.I. (-0.47; -0.06), *p*=0.004], total cholesterol [β=-0.25, 95% C.I. (-0.45; -0.04), *p*=0.05], total cholesterol/HDL [β=-0.25, 95% C.I. (-0.43; -0.06), *p*=0.04], triglycerides [β=-0.14, 95% C.I. (-0.25; -0.02), *p*=0.05], insulin [β=-1.78, 95% C.I. (-2.87; -0.66), *p*=0.009], ApoB/ApoA [β=-0.08, 95% C.I. (-0.13; -03), *p*=0.004], Visceral adipose tissue (VAT) [β=-91.50, 95% C.I. (-132.60; -48.19), *p*<0.0001] and fat mass [β=-945.56, 95% C.I. (-1,424.42; -441.57), *p*=0.004].

CONCLUSIONS: The use of berberine had no adverse events, supporting its use as a natural alternative to pharmacological therapies in the case of IFG.

Key Words:
Berberine, Impaired fasting blood glucose, Phytosome™, Insulin, Body composition.

Introduction

Berberine is a plant alkaloid that has long been used in Indian Ayurvedic and traditional Chinese medicine[1].

The use of berberine is encouraged in the prevention and management of metabolic and cardiovascular diseases, given its anti-inflammatory and antioxidant activities and neuro- and cardioprotective effects demonstrated *in vitro* and animal models[2,3]. The common factor in many metabolic and cardiovascular diseases is a chronic, low-inflammatory state due to increased oxidative stress and the expression of pro-inflammatory cytokines. Berberine modulates the cellular

Corresponding Author: Clara Gasparri, MSc; e-mail: clara.gasparri01@universitadipavia.it

signaling pathways involved in these processes[3]. Berberine exerts a potent anti-inflammatory effect by reducing the secretion of inflammatory cytokines[4,5].

The hypoglycemic action of berberine is complex and is due to different mechanisms, such as the reduction of intestinal glucose absorption[6], the increase in glucose uptake in the liver and muscles[7], the modulation of the intestinal microbiota[8], the removal of gluconeogenesis, and the stimulation of glycolysis[9].

In pancreatic β-cells, berberine acts with an incretin-like mechanism to stimulate the glucagon-like peptide one receptor (GLP-1), thus leading to an increase in insulin secretion[10]. In muscle cells and adipocytes, berberine increases insulin receptor expression. It triggers 5' adenosine monophosphate-activated protein kinase (AMPK), which in turn activates the translocation of GLUT-4 transporters at the membrane level, resulting in increased glucose absorption[3,11,12].

Berberine is absorbed in the gastrointestinal tract with low efficiency (<1%)[13]. In humans, administration of a single dose of 500 mg generates plasma levels of 0.07±0.1 nM. At the same time, it reaches the maximum concentration of 4.0±2.0 nM after three months of chronic administration at a dosage of 15 mg/kg[14]. The low efficiency of berberine transport in the blood is mainly due to poor oral bioavailability, caused by several factors. First, berberine, at low pH levels, tends to self-aggregate. Secondly, berberine is a substrate of P-glycoprotein (Pg-P) that causes its outflow to the intestinal lumen, further limiting absorption[15]. Berberine is metabolized in the liver, where it undergoes a demethylation reaction in phase I, followed by conjugation with glucuronic acid or sulphuric acid to form phase II metabolites of a polar nature that are readily excreted[13]. The CYP2D6 isoform is the most involved[16].

Berberine is safe in most clinical trials, but in small percentages, berberine has been reported to cause nausea, vomiting, constipation, hypertension, respiratory failure, and paresthesias[17]. Overall, the intake of berberine, in doses of 500-1,000 mg/day, should be considered safe for most subjects. The risk of clinically relevant drug interaction due to the activity of berberine on the expression/activity of some isoenzymes of the CYP450 microsomal system is limited to cyclosporin and warfarin[3].

Berbevis™ is a new dietary ingredient consisting of berberine phospholipids. It is characterized by an optimized bioabsorption profile compared to unformulated berberine. The bio-absorption is optimized through the formulation of *Berberis aristata* extract with a specific Phytosome™ that increases its bio-accessibility and tolerability.

The ability of the Berberine Phytosome™ to positively support metabolic health was evaluated in a clinical study[18] conducted on women with polycystic ovary syndrome (PCOS). After 60 days of administration of two 550 mg tablets of Berbevis™, an improvement in the glycemic, lipid, and insulin resistance profiles was recorded.

Given this background, this study aimed to evaluate the potential benefit of oral intake of Berberine Phytosome™ in improving glycemic and lipid values in subjects with impaired fasting blood glucose (IFG).

Patients and Methods

Population

This study was a randomized, double-blind, placebo-controlled trial; it was conducted in overweight men and women with an IFG status (blood glucose between 6.1-7.0 mmol/L, glycosylated hemoglobin <7.0%), according to the American Diabetes Association[19]. The subjects were recruited at the Dietetic and Metabolic Unit of the "Santa Margherita" Institute, University of Pavia, Italy.

Participants, without a history of cardiovascular disease (CVD) and liver, renal, and thyroid disorders, were not allowed to take any drugs likely affecting glucose or lipid metabolism (oral hypoglycemic agents and statins). Subjects with a habit of smoking and drinking more than two standard alcoholic beverages/day (20 g of alcohol/day) were excluded from the study. Physical activity was recorded, and sedentary subjects were admitted to the study. The Ethics Committee of the University of Pavia approved the protocol (ethical code Number: 0912/14122018), and it was registered at ClinicalTrials.gov under the registration number: NCT05031715. Written informed consent was obtained from all the volunteers.

Dietary Supplement

The dietary supplement was associated with one dose before main meals (lunch and dinner) of 550 mg of Berberine Phytosome™ (corresponding to 188 mg berberine). Subjects received the active or placebo for eight weeks.

Indena SpA (Milan, Italy) provided the tablets containing 550 mg of Berberine Phytosome™

(Berbevis™, Indena SpA, Milan, Italy) and the placebo. The latter was identical to active tablets in terms of size, shape, color, odor, and taste. Active and placebo film-coated tablets had a similar composition to inactive food-grade components. According to the Food Supplement European Regulation, before release, the film-coated tablets were verified for appearance, mass (average and uniformity), high-performance liquid chromatographic (HPLC) content of active compounds, disintegration time, and microbiological quality.

Compliance with the supplementation regimen was defined as the number of tablets taken by each subject, divided by the number of tablets that should have been taken throughout the study.

Each treatment group was administered with indistinguishable products, with subjects assigned to each group according to a coded (A or B) block randomization table prepared by an independent statistician. Investigators were blinded to the randomization table, the code assignments, and the procedure. Independent of supplementation, the subjects followed a similar low-energy diet.

Adverse Events

All reports of any adverse events, by subjects and members of the research staff were registered. Moreover, routine blood biochemistry parameters (creatinine and liver function) were evaluated at the start and end of supplementation.

Glycemic and Lipidic Parameters

The glycemic and lipidic parameters were assessed at baseline (t_0, start of the study), after 30 days (t_1), and after 60 days (t_2, end of treatment). Blood samples were immediately centrifuged and frozen at -80°C until assayed. Fasting blood glucose (FBG), total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglyceride (TG) levels were measured by the automatic biochemical analyzer (Hitachi 747, Tokyo, Japan). The serum concentration of hemoglobin A1c (HbA1c) was determined by the HPLC method by an automated HbA1c analyzer (Tosoh HLC-723G7, Japan)[20]. The serum insulin (expressed as pmol/L) was measured by a double antibody RIA (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden). The low detection limit was 10.7 pmol, and the intra- and inter-assay coefficients of variation were below 6%. Insulin resistance was evaluated after 12 hours of fasting. Furthermore, the subjects refrained from physical exercise for 48 hours before the blood sampling. The Homeostasis Model Assessment (HOMA) measured insulin resistance[21].

Anthropometric Measurements and Dietary Counseling

Anthropometric measurements at baseline (t_0, start of the study), after 30 days (t_1), and after 60 days (t_2, end of supplementation) were assessed. A standardized technique was used to measure body weight and height[22], and the body mass index (BMI) was calculated (kg/m^2).

Subjects restricted their daily energy intake by a moderate amount, 3,344 kJ/d less than daily requirements based on WHO criteria[23], with a regimen allowing a careful balance of macronutrients: 25-30% of energy from fat (cholesterol <200 mg), 55-60% of energy from carbohydrates (10% from simple carbohydrates), with 25 g of bran and 15-20% of energy from protein. A 3-day food record of two weekdays and one weekend day was performed during the first and the last week of the study. Participants compiled a three-day food record, including two weekdays and one weekend day during the first and last week of the study. Dietary records were analyzed using a food-nutrient database (Rational Diet, Milan, Italy).

Body Composition

The evaluation of Fat-Free Mass (FFM), Fat Mass (FM), gynoid, and android fat distribution was carried out through the dual-energy X-ray absorptiometry (DXA) with the use of a Lunar Prodigy DXA (GE Medial System, Boston, Massachusetts, United States). The *in vivo* coefficients of variants (CVs) were 0.89% and 0.48% for whole body fat (FM) and FFM, respectively.

Visceral adipose tissue volume (VAT) was estimated using a constant correction factor (0.94 g/cm^3). The software automatically places a quadrilateral box, which represents the android region, outlined by the iliac crest and with a superior height equivalent to 20% of the distance from the top of the iliac crest to the base of the skull[24].

Primary and Secondary Endpoints

The primary endpoint was glycemia, while secondary endpoints included: total cholesterol, HDL, total cholesterol/HLD, LDL, LDL/HDL, triglycerides, insulin, glycated hemoglobin, HOMA, ApoA, ApoB, ApoB/ApoA, androgen suppression treatment (AST), alternative lengthening of telomeres (ALT), gamma-glutamyl transferase (GGT), creatinine, waist circumference, VAT, fat,

and lean mass. All the endpoints were collected at three different time points (i.e., t_0, t_1, and t_2).

Statistical Analysis

Differences between groups at baseline were investigated in each continuous variable by using a *t*-test for independent data in normally distributed variables or the Wilcoxon test in non-normally distributed ones and using Chi-squared in categorial variables. Linear mixed models (LMM) were fitted with time, group, and the interaction time*group as a fixed effect, including a random result in the form of a subject to take into account for intra-subject correlation produced by the repeated measurements[25]. This allowed for the evaluation of statistically significant changes over time for primary and secondary endpoints within and between the two groups. All the models were adjusted for age, sex, and BMI. The normality of residuals for each fitted model were assessed both graphically and *via* the Shapiro-Wilk test. In the case of non-normality of LMM residuals, empirical bootstrap with 5,000 bootstrapped replicates was applied to estimate non-parametric 95% C.I.s and *p*-values based on distribution's quantiles[26]. The statistical significance is fixed as *p*-value ≤ 0.05.

Benjamini-Hochberg correction, fixing the False Discovery Rate (FDR) at $\alpha \leq 0.05$, was used to account for multiple comparisons[27].

In the two groups at both t_0 and at t_2, Pearson's pairwise partial correlations, adjusted for sex and age, were computed between i) the primary endpoint and each secondary endpoint, ii) LDL and each secondary endpoint, iii) VAT and each secondary endpoint, iv) BMI and each secondary endpoint, v) HOMA and each secondary endpoint and vi) fat mass and each secondary endpoint.

Descriptive statistics are reported as Mean ± Standard Deviation (SD). All the analysis was performed on R 3.5.1 statistical software using the nlme and stats packages (R Foundation for Statistical Computing, Vienna, Austria)[28,29].

Results

A total of 49 subjects, 28 females and 21 males, with a mean (±SD) age of (59.5±7.83), were randomly assigned to the supplemented group (n=24) and placebo (n=25), as reported in Table I. No statistically significant differences were observed between the supplemented and placebo groups at baseline parameters.

Table II reports the descriptive statistics regarding mean±SD for each endpoint measured in the two groups at t_0, after 30 days (t_1), and after 60 days (t_2).

Results of the within-group and between-group differences, adjusted for sex, age, and BMI, are reported respectively in Table III and Table IV, both for the primary and the secondary endpoints.

In regards to the within-group differences after multiple testing corrections (Table III) , results showed a statistically significant decrease in glycemia [β=-0.37, 95% C.I. (-0.44; -0.29), $p<0.0001$], total cholesterol [β=-0.21, 95% C.I. (-0.39; -0.02), $p=0.05$], total cholesterol/HDL [β=-0.21, 95% C.I. (-0.37; -0.05), $p=0.02$], LDL/HDL [β=-0.12, 95% C.I. (-0.20; -0.04), $p=0.01$], insulin [β=-2.64, 95% C.I. (-3.44; -1.84), $p<0.0001$], HOMA [β=-0.91, 95% C.I. (-1.24; -0.57), $p<0.0001$], ApoB [β=-4.87, 95% C.I. (-8.73; -0.70), $p=0.04$], ApoB/ApoA [β=-0.06, 95% C.I. (-0.09; -0.03), $p=0.0003$], ALT [β=-1.17, 95% C.I. [-2.07; -0.26], $p=0.02$), waist circumference [β=-1.42, 95% C.I. (-2.10;-0.74), $p=0.0003$], VAT [β=-93.21, 95% C.I. (-127.11; -59.30), $p<0.0001$], and fat mass [β=-1,038.10, 95% C.I. (-1,408.94; -667.27), $p<0.0001$]. While in the placebo group, after multiple testing correction, the results showed after 60 days a statistically significant decrease of HOMA [β=-0.46, 95% C.I. (-0.70; -0.21), $p=0.01$] and glycated hemoglobin [β=-0.14, 95% C.I. (-0.24; -0.05), $p=0.01$], after 60 days of supplementation.

In regards to the between-group differences (Table IV), a statistically significant interac-

Table I. Mean (SD) at baseline (t_0) for age, BMI, and sex and *p*-value of the difference between the two groups.

	Supplemented (n = 24) mean (SD)	Placebo (n = 25) mean (SD)	*p*-value
Age (y)	58.83 (8.47)	60.20 (7.26)	0.55
BMI (Kg/m^2)	30.16 (3.43)	29.47 (3.09)	0.60
Sex, N (%)			1
Female	14 (56.00)	14 (58.33)	
Male	10 (44.00)	11 (41.67)	

BMI: Body Mass Index.

Table II. Descriptive statistics for each endpoint measured in the two groups at baseline (t_0), after 30 days (t_1) and after 60 days (t_2).

	Supplemented group (n = 24) mean (SD)			Placebo group (n = 25) mean (SD)		
	t_0	t_1	t_2	t_0	t_1	t_2
Primary endpoint						
Glycemia (mmol/L)	6.15 (0.45)	5.49 (0.58)	5.42 (0.46)	6.22 (0.36)	6.16 (0.34)	5.97 (1.16)
Secondary endpoint						
Total Cholesterol (mmol/L)	5.23 (1.17)	4.77 (0.91)	4.82 (0.90)	5.47 (0.52)	5.53 (0.44)	5.55 (0.46)
HDL (mmol/L)	1.47 (0.38)	1.46 (0.34)	1.52 (0.36)	1.52 (0.47)	1.51 (0.44)	1.51 (0.47)
Total Cholesterol/HDL	3.71 (0.99)	3.39 (0.81)	3.28 (0.81)	3.90 (1.15)	3.95 (1.06)	3.97 (1.11)
LDL (mmol/L)	3.22 (1.00)	2.92 (0.78)	3.03 (0.81)	3.35 (0.53)	3.35 (0.56)	3.32 (0.44)
LDL/HDL	2.32 (0.94)	2.09 (0.67)	2.08 (0.69)	2.40 (0.80)	2.42 (0.86)	2.38 (0.74)
Triglycerides (mmol/L)	1.35 (0.59)	1.19 (0.54)	1.17 (0.40)	1.23 (0.52)	1.32 (0.49)	1.33 (0.50)
Insulin (mcIU/ml)	18.03 (11.23)	14.65 (9.60)	12.75 (8.33)	13.67 (9.10)	12.83 (9.07)	11.92 (7.89)
HOMA (pt)	5.06 (3.40)	3.73 (2.77)	3.24 (1.98)	3.79 (2.55)	3.50 (2.53)	2.88 (2.05)
Glycated haemoglobin (%)	5.87 (0.57)	5.71 (0.39)	5.82 (0.47)	6.00 (0.57)	5.90 (0.53)	5.72 (0.58)
ApoA (mg/dl)	146.67 (29.72)	147.00 (21.82)	154.88 (21.94)	151.36 (26.07)	152.48 (30.29)	150.20 (26.93)
ApoB (mg/dl)	110.58 (32.56)	98.79 (26.22)	100.83 (23.76)	120.32 (22.98)	127.00 (25.97)	124.24 (24.07)
ApoB/ApoA	0.78 (0.28)	0.68 (0.19)	0.66 (0.19)	0.82 (0.22)	0.87 (0.25)	0.86 (0.24)
AST (IU/l)	21.38 (5.94)	21.00 (5.00)	19.88 (5.33)	20.75 (5.85)	21.40 (4.45)	20.76 (5.15)
ALT (IU/l)	23.00 (7.11)	21.62 (6.78)	20.67 (6.57)	22.20 (7.29)	22.52 (5.89)	22.56 (10.40)
G-GT (U/l)	24.33 (6.63)	23.29 (7.39)	23.88 (7.94)	22.68 (5.42)	21.40 (7.19)	23.32 (10.15)
Creatinine (mg/dl)	0.84 (0.16)	0.85 (0.15)	0.84 (0.18)	0.83 (0.11)	0.81 (0.13)	0.81 (0.14)
Waist Circumference (cm)	105.40 (12.34)	102.73 (12.11)	102.56 (12.29)	100.32 (9.33)	99.88 (9.38)	107.08 (27.96)
VAT (g)	1,622.54 (709.92)	1,437.08 (620.40)	1,436.12 (652.77)	1,361.96) (555.66	1,337.16 (549.01)	1,358.52 (544.91)
Fat Mass (g)	35,696.29 (13,358.39)	34,496.33 (12,826.43)	33,620.08 (13,081.70)	28,222.04 (10,266.76)	28,034.72 (10,342.97)	28,036.96 (10,359.15)
Lean Mass (g)	46,277.42 (8,603.81)	46,058.17 (8,416.40)	46,035.21 (8,783.83)	432,23.32 (6,902.46)	42,885.68 (7,117.47)	42,867.36 (7,116.76)

VAT: visceral adipose tissue; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; ApoB: Apolipoprotein B; ApoA: Apolipoprotein A; ALT: alanine aminotransferase; AST: aspartate aminotransferase; G-GT: Gamma-Glutamyl Transferase.

tion between time and group, meaning that the change in score over time is different for each group, was observed for glycemia [β=-0.24, 95% C.I. (-0.47; -0.06), p=0.004], total cholesterol [β=-0.25, 95% C.I. (-0.45; -0.04), p=0.05], total cholesterol/HDL [β=-0.25, 95% C.I. (-0.43; -0.06), p=0.04], triglycerides [β=-0.14, 95% C.I. (-0.25; -0.02), p=0.05], insulin [β=-1.78, 95% C.I. (-2.87; -0.66), p=0.009], ApoB/ApoA [β=-0.08, 95% C.I. (-0.13; -0.03), p=0.004], VAT [β=-91.50, 95% C.I. (-132.60; -48.19), p<0.0001] and fat mass [β=-945.56, 95% C.I. (-1,424.42; -441.57), p=0.004].

Results of all Pearson's pairwise partial correlations adjusted for sex and age are reported in **Supplementary Table I**.

Interestingly, high, positive statistically significant pairwise correlations were observed at t_0 between glycemia and insulin (r=0.62, p=0.002), glycemia and HOMA (r=0.68, p=0.0005), LDL and LDL/HLD (r=0.85, p<0.0001), LDL and ApoB (r=0.97, p<0.0001) LDL and ApoB/ApoA (r=0.81, p<0.0001), VAT and triglycerides (r=0.65, p=0.001), VAT and waist circumference (r=0.82, p<0.0001), VAT and fat mass (r=0.65, p=0.001), BMI and insulin (r=0.62, p=0.002), BMI and HOMA (r=0.62, p=0.002), BMI and waist circumference (r=0.85, p<0.0001), BMI and VAT (r=0.75, p<0.0001), BMI and fat mass (r=0.92, p<0.0001), HOMA and insulin (r=1, p<0.0001), HOMA and fat mass (r=0.71, p=0.0002), fat mass and insulin (r=0.73, p=0.0001), fat mass and waist circumference (r=0.74, p<0.0001) in the supplemented group. At t_2 in the supplemented group, high, positive, statistically significant pairwise

Table III. Within-group pre-post supplementation difference for primary and secondary endpoints. Estimate of time (β), 95% confidence interval (CI) and the adjusted *p*-value of the null hypothesis of a no effect are reported for the two groups.

	Supplemented group		Placebo group	
	Time β [95% CI]	*p*-value adjusted	Time β [95% CI]	*p*-value adjusted
Primary endpoint				
Glycemia (mmol/l)	-0.37 [-0.44; -0.29]	**< 0.0001**	-0.13 [-0.28; 0.09]	0.57
Secondary endpoint				
Total Cholesterol (mmol/l)	-0.21 [-0.39; -0.02]	**0.05**	0.04 [-0.03; 0.11]	0.60
HDL (mmol/l)	0.03 [-0.02; 0.07]	0.32	-0.004 [-0.03; 0.02]	0.82
Total Cholesterol/HDL	-0.21 [-0.37; -0.05]	**0.02**	0.03 [-0.05; 0.12]	0.69
LDL (mmol/l)	-0.09 [-0.21; 0.03]	0.19	-0.01 [-0.06; 0.03]	0.73
LDL/HDL	-0.12 [-0.20; -0.04]	**0.01**	-0.01 [-0.07; 0.05]	0.82
Triglycerides (mmol/l)	-0.09 [-0.18; 0.002]	0.09	0.05 [-0.03; 0.12]	0.58
Insulin (mcIU/ml)	-2.64 [-3.44; -1.84]	**< 0.0001**	-0.87 [-1.78; -0.04]	0.26
HOMA (pt)	-0.91 [-1.24; -0.57]	**< 0.0001**	-0.46 [-0.70; -0.21]	**0.01**
Glycated haemoglobin (%)	-0.02 [-0.11; 0.07]	0.67	-0.14 [-0.24; -0.05]	**0.01**
ApoA (mg/dl)	4.10 [-0.38; 8.78]	0.10	-0.58 [-3.47; 2.31]	0.82
ApoB (mg/dl)	-4.87 [-8.73; -0.70]	**0.04**	1.96 [-2.50; 5.68]	0.60
ApoB/ApoA	-0.06 [-0.09; -0.03]	**0.0003**	0.02 [-0.02; 0.05]	0.60
AST (IU/l)	-0.75 [-1.52; 0.02]	0.09	-0.16 [-1.54; 1.16]	0.90
ALT (IU/l)	-1.17 [-2.07; -0.26]	**0.02**	0.18 [-2.17; 2.11]	0.82
G-GT (U/l)	-0.23 [-1.06; 0.60]	0.67	0.32 [-1.48; 2.07]	0.82
Creatinine (mg/dl)	0.001 [-0.01; 0.02]	0.88	-0.005 [-0.02; 0.009]	0.71
Waist Circumference (cm)	-1.42 [-2.10; -0.74]	**0.0003**	3.38 [-1.53; 7.14]	0.55
VAT (g)	-93.21 [-127.11; -59.30]	**< 0.0001**	-1.72 [-14.73; 11.29]	0.83
Fat Mass (g)	-1,038.10 [-1,408.94; -667.27]	**< 0.0001**	-92.54 [-189.65; 4.57]	0.26
Lean Mass (g)	-121.10 [-431.72; 189.52]	0.53	-177.98 [-509.52; 154.91]	0.60

In bold are indicated the significant results ($p \leq 0.05$). In bold are indicated the significant results ($p \leq 0.05$). VAT: visceral adipose tissue; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; ApoB: Apolipoprotein B; ApoA: Apolipoprotein A; ALT: alanine aminotransferase; AST: aspartate aminotransferase; G-GT: Gam-ma-Glutamyl Transferase.

correlations were observed between LDL and total cholesterol (r=0.89, p<0.0001), LDL and LDL/HDL (r=0.79, p<0.0001), LDL and ApoB (r=0.94, p<0.0001), LDL and ApoB/ApoA (r=0.80, p<0.0001), VAT and waist circumference (r=0.81, p<0.0001), VAT and fat mass (r=0.69, p=0.0003), BMI and waist circumference (r=0.86, p<0.0001), BMI and VAT (r=0.77, p<0.0001), BMI and fat mass (r=0.91, p<0.0001), HOMA and insulin (r=0.90, p<0.0001), fat mass and insulin (r=0.66, p=0.0009), fat mass and waist circumference (r=0.79, p<0.0001). As for the control group, high, positive statistically significant pairwise correlations were observed at t_0 between VAT and waist circumference (r=0.79, p<0.0001), VAT and fat mass (r=0.80, p<0.0001), BMI and VAT (r=0.79, p<0.0001), BMI and fat mass (r=0.89, p<0.0001), HOMA and insulin (r=1, p<0.0001), while a t_2, high, positive statistically significant pairwise correlations were observed between VAT and fat mass (r=0.79, p<0.0001), BMI and VAT (r=0.77, p<0.0001), BMI and fat mass (r=0.88, p<0.0001), and HOMA and insulin (r=0.90, p<0.0001).

Discussion

The present study demonstrates the efficacy of a two-month-long supplementation of Berberine Phytosome™ on the metabolic profile and body composition of overweight subjects with IFG. In particular, a statistically significant improvement in the primary endpoint has been observed in the supplemented group; in fact, glycemia was significantly reduced, and considering the glycemic profile, insulin decreased. The lipid profile also improved in the supplemented group; total cholesterol levels, total/HDL cholesterol ratio, and triglycerides showed a statistically signifi-

Table IV. Between-group pre-post supplementation difference for primary and secondary endpoints. Estimate of time*treatment (β), 95% confidence interval (CI) and the adjusted *p*-value of the null hypothesis of a no effect are reported.

	Time*group β [95% CI]	*p*-value adjusted
Primary endpoint		
Glycemia (mmol/l)	-0.24 [-0.47; -0.06]	**0.004**
Secondary endpoint		
Total Cholesterol (mmol/l)	-0.25 [-0.45; -0.04]	**0.05**
HDL (mmol/l)	0.03 [-0.02; 0.09]	0.33
Total Cholesterol/HDL	-0.25 [-0.43; -0.06]	**0.04**
LDL (mmol/l)	-0.08 [-0.21; 0.05]	0.33
LDL/HDL	-0.11 [-0.22;- 0.003]	0.11
Triglycerides (mmol/l)	-0.14 [-0.25; -0.02]	**0.05**
Insulin (mcIU/ml)	-1.78 [-2.87; -0.66]	**0.009**
HOMA (pt)	-0.45 [-0.86; -0.04]	0.07
Glycated haemoglobin (%)	0.11 [-0.01; 0.25]	0.12
ApoA (mg/dl)	4.68 [-0.92; 10.40]	0.15
ApoB (mg/dl)	-6.83 [-12.27; -0.79]	0.7
ApoB/ApoA	-0.08 [-0.13; -0.03]	**0.004**
AST (IU/l)	-0.56 [-2.14; 1.17]	0.57
ALT (IU/l)	-1.35 [-3.55; 1.29]	0.33
G-GT (U/l)	-0.55 [-2.57; 1.56]	0.64
Creatinine (mg/dl)	0.01 [-0.01; 0.03]	0.64
Waist Circumference (cm)	-4.80 [-8.93; 0.30]	0.11
VAT (g)	-91.50 [-132.60; -48.19]	**< 0.0001**
Fat Mass (g)	-945.56 [-1,424.42; -441.57]	**0.004**
Lean Mass (g)	56.88 [-337.88; 451.63]	0.77

In bold are indicated the significant results ($p \leq 0.05$). VAT: visceral adipose tissue; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; ApoB: Apolipoprotein B; ApoA: Apolipoprotein A; ALT: alanine aminotransferase; AST: aspartate aminotransferase; G-GT: Gam-ma-Glutamyl Transferase.

cant reduction. Furthermore, ApoB/ApoA ratio decreased, which is an accurate risk factor for cardiovascular disease: the lower the ApoB/ApoA ratio, the lower the risk[30].

The results of our study agree with previous research, which has demonstrated that berberine was effective in modulating the glycemic profile. Zhang et al[31] showed that berberine increases insulin receptor expression and improves glucose utility both in *in vitro* and *in vivo* models[31]. The authors confirmed the activity of berberine in humans and its relationship with the glucose-lowering effect[8]. Pérez-Rubio[32] demonstrated that administration of 500 mg berberine three times a day leads to remission of metabolic syndrome and decreases in waist circumference, systolic blood pressure, triglycerides, and total insulin secretion, with an increase in insulin sensitivity, in subjects with metabolic syndrome. Nutraceutical supplements containing berberine, red yeast rice, coenzyme Q10, folic acid, and chrome were effective in improving lipid pattern and glucose levels in individuals with pre-hypertension. A more significant reduction of total cholesterol, LDL cholesterol, and glycemia was observed in the treatment group[33].

Moreover, berberine was effective in improving blood lipid levels (triglycerides, total cholesterol, LDL decreased, and HDL increased in the treatment group) in subjects with mild hyperlipidemia[34].

Oral administration of berberine significantly reduced body weight, and improved metabolic profile for lipid and glucose in patients with non-alcoholic fatty liver disease[35].

Another significant result of the present study is the positive changes observed in the body composition of the subjects supplemented with Berberine Phytosome™. Specifically, VAT and fat mass were significantly reduced.

These results agree with a previous study in which the administration of 550 mg of berberine twice daily in PCOS women led to a statistically significant reduction of BMI, waist circumference, VAT, and fat mass, in addition to the improvements in HOMA values[18]. Increasing evidencencreasing evidence has shown that natural plant-based products may play a role in PCOS management. The

aim of this one-group pretest–post-test explanatory study was to evaluate, in normal–overweight PCOS women with normal menses, the effectiveness of berberine on: Insulin Resistance (IR). The decrease in VAT is a positive result since it is associated with metabolic syndrome and cardiovascular disease, and it is also an independent risk factor of all-cause mortality[36]. The management of VAT is a noteworthy result; in fact, VAT is considered equal to an endocrine organ capable of creating significant interactions between the brain, the liver, the skeletal muscle, the heart, and blood vessels[37].

All these results obtained (the statistically significant interaction between time and group on glycemia, total cholesterol, total cholesterol / HDL, triglycerides, insulin, ApoB / ApoA, VAT, fat mass, and the high positive statistically significant pairwise correlations observed), underline the pleiotropic activity of berberine on the metabolic profile. As discussed in a recent review by Feng et al[9], the significant inhibitory potency of Berberine against both α-amylase and α-glucosidase represents a possible explanation for its pleiotropic effect.

Laboratory tests were performed at baseline and end of treatment to test the safety of the product. No relevant adverse effects emerged. Thus, the present study has confirmed that berberine is well-tolerated with good compliance.

Limitations

The main limitations of this study are the small sample size and the relatively short duration of the intervention, which is only two months. Therefore, further studies with larger sample sizes and a longer period of intervention are needed to investigate further potential effects of berberine on the glycemic profile.

Conclusions

The results of this study are encouraging. In fact, in the supplemented group, both glycemia and insulin decreased. Moreover, the lipid profile improved, and total cholesterol levels, total/HDL cholesterol ratio, and triglycerides showed a statistically significant reduction. Even the ApoB/ApoA ratio decreased.

The administration of Berberine Phytosome™ did not result in side effects, supporting its potential use as a natural alternative to pharmacological therapies in the case of IFG.

Conflict of Interest

Authors GPet, PA, DA, and AR were employed by Indena SpA (Milan, Italy). The remaining authors declare that the research was conducted without any commercial or financial relationships that could be construed as a po-tential conflict of interest.

Authors' Contribution

Conceptualization, MR; methodology, MR and AR; validation, GPet, PA, DA, and AR; formal analysis, TF, and LB; data curation, ZP, FM, AT, and AC; writing-original draft preparation, MR; writing-review and editing, CG and GPer; project administration, MR. All authors have read and agreed to the published version of the manu-script.

Funding

This research did not receive external funding.

Ethics Approval

The study was conducted following the Declaration of Helsinki and approved by the Ethics Committee of the University of Pavia (ethical code Number: 0912/14122018).

Informed Consent

Informed consent was obtained from all subjects involved in the study.

ORCID ID

M. Rondanelli: 0000-0001-8336-4851
C. Gasparri: 0000-0002-1088-6648
G. Petrangolini: 0000-0001-6681-7329
P. Allegrini: 0000-0002-4380-9577
T. Fazia: 0000-0002-9577-8450
L. Bernardinelli: 0000-0002-7551-6664
G. Peroni: 0000-0002-1632-1787
A. Tartara: 0000-0002-7480-0153
A. Riva: 0000-0003-2819-943X

References

1) Chander V, Aswal J, Dobhal R, Uniyal D. A review on Pharmacological potential of Berberine; an active component of Himalayan Berberis aristata. J Phytopharm 2017; 6: 53-58.
2) Cai Y, Xin Q, Lu J, Miao Y, Lin Q, Cong W, Chen K. A New Therapeutic Candidate for Cardio-vascular Diseases: Berberine. Front Pharmacol 2021; 12: 631100.
3) Cicero AFG, Baggioni A. Berberine and Its Role in Chronic Disease. Adv Exp Med Biol 2016; 928: 27-45.
4) Huang LH, Pan XP, Gong KR, Shao G. Anti-inflammatory effects of three kinds of tradition-

al Mongolian medicine monomer and its combination on LPS-stimulated RAW264.7 macrophages. Eur Rev Med Pharmacol Sci 2016; 20: 950-958.

5) Yu XH, Wang YF, Dai FY, Zhao JH, Li P. The protective effects of Berberine and Hesperidin on inflammatory factor-stimulating cardiac fibroblasts. Eur Rev Med Pharmacol Sci 2019; 23: 5468-5476.
6) Pan GY, Huang ZJ, Wang GJ, Fawcett JP, Liu XD, Zhao XC, Sun JG, Xie YY. The antihyper-glycaemic activity of berberine arises from a decrease of glucose absorption. Planta Med 2003; 69: 632-636.
7) Yin J, Gao Z, Liu D, Liu Z, Ye J. Berberine improves glucose metabolism through induction of glycolysis. Am J Physiol Endocrinol Metab 2008; 294: E148-E156.
8) Zhang H, Wei J, Xue R, Wu JD, Zhao W, Wang ZZ, Wang SK, Zhou ZX, Song DQ, Wang YM, Pan HN, Kong WJ, Jiang JD. Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. Metabolism 2010; 59: 285-292.
9) Feng X, Sureda A, Jafari S, Memariani Z, Tewari D, Annunziata G, Barrea L, Hassan STS, Šmejkal K, Malaník M, Sychrová A, Barreca D, Ziberna L, Mahomoodally MF, Zengin G, Xu S, Nabavi SM, Shen AZ. Berberine in Cardiovascular and Metabolic Diseases: From Mechanisms to Therapeutics. Theranostics 2019; 9: 1923-1951.
10) Li M, Zhou W, Dang Y, Li C, Ji G, Zhang L. Berberine compounds improves hyperglycemia via microbiome mediated colonic TGR5-GLP pathway in db/db mice. Biomed Pharmacother 2020; 132: 110953.
11) Krishan S, Richardson DR, Sahni S. Adenosine monophosphate-activated kinase and its key role in catabolism: structure, regulation, biological activity, and pharmacological activation. Mol Pharmacol 2015; 87: 363-377.
12) Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y, Ye JM, Le CH, Oh WK, Kim CT, Hohnen-behrens C, Gosby A, Kraegen EW, James DE, Kim JB. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insu-lin-resistant states. Diabetes 2006; 55: 2256-2264.
13) Kumar A, Ekavali, Chopra K, Mukherjee M, Pottabathini R, Dhull DK. Current knowledge and pharmacological profile of berberine: An update. Eur J Pharmacol 2015; 761: 288-297.
14) Hua W, Ding L, Chen Y, Gong B, He J, Xu G. Determination of berberine in human plasma by liquid chromatography-electrospray ionization-mass spectrometry. J Pharm Biomed Anal 2007; 44: 931-937.
15) Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech 2009; 2: 231-237.
16) Wang H, Zhu C, Ying Y, Luo L, Huang D, Luo Z. Metformin and berberine, two versatile drugs in treatment of common metabolic diseases. Oncotarget 2017; 9: 10135-10146.
17) Derosa G, Maffioli P, Cicero AFG. Berberine on metabolic and cardiovascular risk factors: an analysis from preclinical evidences to clinical trials. Expert Opin Biol Ther 2012; 12: 1113-1124.
18) Rondanelli M, Riva A, Petrangolini G, Allegrini P, Giacosa A, Fazia T, Bernardinelli L, Gasparri C, Peroni G, Perna S. Berberine Phospholipid Is an Effective Insulin Sensitizer and Improves Metabolic and Hormonal Disorders in Women with Polycystic Ovary Syndrome: A One-Group Pretest-Post-Test Explanatory Study. Nutrients 2021; 13: 3665.
19) Sherwin RS, Anderson RM, Buse JB, Chin MH, Eddy D, Fradkin J, Ganiats TG, Ginsberg HN, Kahn R, Nwankwo R, Rewers M, Schlessinger L, Stem M, Vinicor F, Zinman B. The prevention or delay of type 2 diabetes: American Diabetes Association and National Institute of Diabetes, Digestive and Kidney Diseases [Internet]. Vol. 25, Diabetes Care. Diabetes Care 2002; 26 Suppl 1: S62-S69
20) Terreni A, Paleari R, Caldini A, Ognibene A, Mosca A, Messeri G. Evaluation of the analytic performances of the new HPLC system HLC-723 G7 for the measurement of hemoglobin A1c. Clin Biochem 2003; 36: 607-610.
21) Haffner SM, Kennedy E, Gonzalez C, Stern MP, Miettinen H. A prospective analysis of the HOMA model. The Mexico City Diabetes Study. Diabetes Care 1996; 19: 1138-1141.
22) Frisancho AR. New standards of weight and body composition by frame size and height for as-sessment of nutritional status of adults and the elderly. Am J Clin Nutr 1984; 40: 808-819.
23) World Health Organization. Energy and protein requirements - Report of a Joint FAO/WHO/UNU Expert Consultation. 1985. Available at: https://apps.who.int/iris/handle/10665/39527.
24) Mohammad A, De Lucia Rolfe E, Sleigh A, Kivisild T, Behbehani K, Wareham NJ, Brage S, Mo-hammad T. Validity of visceral adiposity estimates from DXA against MRI in Kuwaiti men and women. Nutr Diabetes 2017; 7: e238.
25) Pinheiro J, Bates D. Mixed-effects models in S and S-PLUS. Springer Science & Business Media, editor, 2006.
26) Rice JA. Mathematical Statistics and Data Analysis - Third Edition. Cengage Learning, 2010.
27) Benjamin Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. Ann Stat 2001; 29: 1165-1188.
28) R Core Team. R: a language and environment for statistical computing [Internet], 2017.
29) José Pinheiro S, Bates D, DebRoy S, Sarkar D, Team R. Linear and Nonlinear Mixed Effects

Models. Available at: https://www.researchgate.net/publication/303803175_NIme_Linear_and_Nonlinear_Mixed_Effects_Models.

30) Walldius G, Jungner I. The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy--a review of the evidence. J Intern Med 2006; 259: 493-519.

31) Zhang Y, Li X, Zou D, Liu W, Yang J, Zhu N, Huo L, Wang M, Hong J, Wu P, Ren G, Ning G. Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. J Clin En-docrinol Metab 2008; 93: 2559-2565.

32) Pérez-Rubio KG, González-Ortiz M, Martínez-Abundis E, Robles-Cervantes JA, Espinel-Bermúdez MC. Effect of berberine administration on metabolic syndrome, insulin sensitivity, and insulin se-cretion. Metab Syndr Relat Disord 2013; 11: 366-369.

33) Mazza A, Schiavon L, Rigatelli G, Torin G, Lenti S. The Effects of a New Generation of Nutraceutical Compounds on Lipid Profile and Glycaemia in Subjects with Pre-hypertension. High Blood Press Cardiovasc Prev 2019; 26: 345-350.

34) Wang L, Peng L yun, Wei G hong, Ge H. Therapeutic Effects of Berberine Capsule on Patients with Mild Hyperlipidemia. Chinese J Integr Tradit West Med 2016; 36: 681-684.

35) Yan HM, Xia MF, Wang Y, Chang XX, Yao XZ, Rao SX, Zeng MS, Tu YF, Feng R, Jia WP, Liu J, Deng W, Jiang JD, Gao X. Efficacy of Berberine in Patients with Non-Alcoholic Fatty Liver Disease. PLoS One 2015; 10: e0134172.

36) Kivimäki M, Kuosma E, Ferrie JE, Luukkonen R, Nyberg ST, Alfredsson L, Batty GD, Brunner EJ, Fransson E, Goldberg M, Knutsson A, Koskenvuo M, Nordin M, Oksanen T, Pentti J, Rugulies R, Shipley MJ, Singh-Manoux A, Steptoe A, Suominen SB, Theorell T, Vahtera J, Virtanen M, Westerholm P, Westerlund H, Zins M, Hamer M, Bell JA, Tabak AG, Jokela M. Overweight, obesity, and risk of cardiometabolic multimorbidity: pooled analysis of individual-level data for 120 813 adults from 16 cohort studies from the USA and Europe. Lancet Public Heal 2017; 2: e277-e285.

37) Fang H, Berg E, Cheng X, Shen W. How to best assess abdominal obesity. Curr Opin Clin Nutr Metab Care 2018; 21: 360-365.