Metabolic Effects of an Inositol-Resveratrol Nutraceutical Combination in Non-Diabetic Overweight/Obese Subjects with Altered Glucose Tolerance

Guarino G[1], Della Corte T[*1], Letizia M[1], Fontana S[1], Strollo F[2] and Gentile S[3]

[1]Department of Clinical and Experimental Medicine, Campania University "Luigi Vanvitelli", Naples, Italy
[2]Endocrinology and Metabolism, Elle-di, Rome, Italy
[3]Diabetes Unit AID Stabia, Castellammare di Stabia, and Nefrocenter Reserach Nerwork, Naples, Italy

[*]Corresponding author: Della Corte T, Department of Clinical and Experimental Medicine, Campania University "Luigi Vanvitelli", Naples, Italy, E-mail: tere.dellacorte@gmail.com

Summary

Insulin resistance (IR) is almost constistently associated with overweight or obesity in both diabetic and non-diabetic subjects. Nutraceutical compounds improving insulin resistance (IR) may be useful in prevention and treatment of cardiovascular (CV) risk factors in metabolic diseases. D-Chiro-inositol (DCI) and Myo-inositol (MY), two isomers of a C6 poliol called inositol, are involved in the transduction pathway of the insulin signal, thus contributing to glucose regulation, and have therefore been studied in various metabolic conditions characterized by IR. Opposite to that, Resveratrol (R), a compound known to counteract oxidative stress mediated events eventually leading to IR and strongly involved in aging processes, has been mainly tested in preclinical or *in vitro* studies. The combination of those three molecules might then prove to be of great help against obesity and associated dyslipidemia / IR.

The aim of our 24-week, randomized, double-blind study was to verify whether and to what extent a fixed MY-DCI-R association can affect IR and abdominal fat in pre-diabetic patients with a body mass index (BMI) $\geq$ 26 kg/m^2 and hyperlipidemia as compared to placebo (P). Biochemical, anthropometric and bioimpedance-derived parameters and clinical benefits were regularly recorded in addition to any side/toxic effects. As compared to placebo, combined MY-DCI-R significantly improved CV risk factors including body weight, visceral/trunk fat amount, IR, systolic/diastolic blood pressure, circulating lipids and uric acid levels.

Keywords: Inositol; Resveratrol; Neutraceuticals; Insulin Resistance; Metabolism; Prediabetes; IFG; IGT; Myo-Inositol; Reversatrol; Nutracautical; Obesity

Introduction

Insulin resistance (IR) is a low insulin sensitivity condition characterized by sub-optimal biological response to normal amounts of insulin. It may involve several organs and tissues (including liver, skeletal muscle and adipose tissue) or a single cell type [1].

It may be a consequence of excess visceral adipose tissue, high fat diet, poor physical activity, cigarette smoking and drugs (glucocorticoids, thiazide diuretics, beta-blockers above all) [1], mainly acting through high levels of circulating free fatty acids (the so called "lipotoxic effect") [2,3], hyperglycemia (the so called "glycotoxic effect") [4], and inflammatory cytokines secreted by activated macrophages *per se* or in combination with abdominal adipocytes [5].

Inositol is a widely spread carbocyclic poliol presenting within the organism as different isomers, the most frequent among which is Myo-inositol (MY). The latter is epimerase-converted into the active metabolite D-Chiro-inositol (DCI) within insulin-dependent and insulin-sensitive structures including liver, muscles and adipose tissue, as well as, in organs endowed with a high-energy metabolism, i.e. heart and brain. [6-9]. At the cell level both MY and DCI act as second messengers for insulin through two independent mechanisms, i.e. glucose uptake and glycogen synthesis, respectively [10].

Given their pivotal role in regulating metabolic and hormonal signaling pathways, inositol isomers are more and more utilized as supplements in clinical settings typically characterized by insulin resistance, like Polycystic Ovary Syndrome (PCOS) [11] - which is known to be associated with inositol-phospho-glycan deficiency [12] - and the metabolic syndrome [9,13]. In addition to that, gestational diabetes (GDM), i.e. an altered glucose handling condition first detected in pregnancy, also benefits from their insulin-sensitizing effect in terms of both prevention and treatment [14-18] and, based on preliminary data, the same seems to hold true for IR in obese male children [19].

Another interesting substance produced by several plants and especially in *polygonum capsulatum* roots and grape skin is Resveratrol (3,5,4'-trihydroxy-trans-stilbene) (RSV), a non-flavonoid phenol from the phytoalexin family, known to be very active against pathogens including bacteria and fungi [20] and to be endowed with an anti-tumor [21] anti-inflammatory [22], and blood-thinning activity eventually limiting the onset of thrombotic plaques [23]. Several *in vitro* studies documented powerful anti-aging effects thanks to its relevant antioxidant activity within the skin negatively affecting collagenase or matrix metalloproteinase-16 production [24,25]. In addition, by acting mainly "upstream" of the reaction through copper ion chelation-dependent catalytic effect inactivation, RSV proves to be a more powerful antioxidant than flavonoids and might be beneficial by hindering LDL peroxidation *in vivo* too [26]. However, its main anti-oxidant effect is mediated by the activation of SIRT-1 gene encoding a histone deacetilase (SIRTUIN-1) [19,27,28], which might help prevent and treat chronic degenerative conditions including cardio-vascular diseases, metabolic syndrome and or obesity, osteoporosis, diabetes, cancer, as well as, several inflammatory and neuro-degenerative conditions.

RSV is virtually insoluble in water and displays a low dissolution rate from the solid pharmaceutical oral formulation. This involves unfavorable pharmacokinetic properties in humans, with low bioavailability and a short plasma half-life [29,30], partly offset, in fact, by its strong lipophilia-dependent ability to easily cross plasma membranes [31,32]. According to some studies RSV bioavailability may be improved by embedding trans-resveratrol isomers in a magnesium hydroxide matrix allowing the two components to keep their chemical identity while maintaining RSV dissolved in a liquid-like structure [29-33].

Based on the above mentioned large body of evidence on their complementary biological activity, we evaluated the effects of associated MY, DCI and RSV (MCR) on a large group of overweight or obese subjects with documented pre-diabetes and insulin-resistance kept at a low-calorie diet during a 24-week placebo-controlled study.

We preferred to recruit severely insulin-resistant subjects with pre-diabetes and to exclude people with diabetes to rule out any biases potentially depending on interfering glucose handling mechanisms causing glucotoxic effects.

The primary endpoint was the extent and distribution of fat tissue, secondary endpoints were: (1) anthropometric and biochemical parameters; (2) blood pressure levels; (3) uric acid levels; (4) MCR safety, tolerability, and drop out rate; and (5) durability of MCR effects.

Tested Product

Kirocomplex® is a product for human use (source: https://www.fogliettoillustrativo.net/bugiardino/kirocomplex-20cpr-927126870; registration number: AIC code: 927126870 and EAN code: 9271268706), registered by s&r Farmaceutici spa Company, Italy) notified to the Ministry of Health in agreement with Italian law # 169/2004 and containing active ingredients (*D-Chiro-Inositol, Myo-Inositol,* and *Polygonum cuspidatum extract on Magnesium hydroxide, titrated to at least 30% Resveratrol*) included in the positive list of nutraceuticals within the food supplements / diet supplements and special foods category with all of its food grade excipients. In greater detail, a tablet of Kirocomplex® (K) contains: D-Chiro-Inositol 500 mg; Myo-Inositol 200 mg, Revifast 80 mg (i.e. resveratrol 48 mg), folic acid 200 mg, D-Vitamin 12.5 mg, and manganese 5 mg.

Materials and Methods

Patients, Diet, and Measures

This was a double-blind, placebo-controlled study conducted in accordance with the Helsinki Declaration and formally approved by the Ethics Committee of the University of Campania "*Luigi Vanvitelli*" (Trial registration number 119, 10.01.2018). A sample size of 45 pairs of subjects meeting the inclusion criteria was calculated based on Green's [34] regression-specific recommendations but 60 pairs were enrolled in the study to compensate for any possible drop-outs.

Inclusion Criteria were:

1. Age between 18 and 70 years;
2. Overweight (BMI 25-29.9 Kg/m²) / obesity (BMI >30 kg/m²) according to WHO definition [35]
3. Impaired fasting glucose (IGF) and/or impaired glucose tolerance to OGTT (IGT), being IFG defined as a fasting 100 to 125 mg/dL blood glucose level, and IGT as a 140 to 199 mg/dL blood glucose level attained 2 hours after the administration of a standard 75-g oral glucose tolerance test (OGTT) according to ADA criteria [36]
4. Insulin resistance index (HOMA-R) >2.5);
5. Altered lipid profile (total cholesterol >200 mg/dl; LDL-cholesterol >100 mg/dl);
6. No previous Bariatric surgery
7. Agreement to participate in the study.

The Exclusion Criteria were:

1. Type 1 or 2 diabetes;
2. Treatment with oral or injectable hypoglycemic agents;
3. Known hypersensitivity / intolerance to D-Chiro-Inositol, Myo-Inositol and Reversatrol;
4. Known, severe kidney and/or liver diseases or malignancies;
5. Contraceptive pill utilization;

6.   Planned or ongoing pregnancy or breastfeeding;
7.   Polycystic Ovary Syndrome (PCOS).
8.   Being on glucose-lowering or cholesterol-lowering medications;
9.   Any previous acute cardiovascular events;
10. Any previous bariatric surgery;

A total of 120 consecutive subjects meeting the above mentioned criteria were enrolled during a three-month period (Figure 1) and randomly assigned to either active treatment (Treatment Group; 60 subjects on Kirocomplex®, "T", 1 tablet/day) or placebo (Placebo Group; 60 subjects, "P", 1 tablet/day) packed in identical containers endowed with a serial code provided by a random paired number generator. Placebo tablets were prepared by the hospital pharmacy, contained only folic acid 200 mg, D-Vitamin 12,5 mg, and manganese 5 mg and were indistinguishable from the others in terms of size, color, smell and taste. This way each component of each pair of subjects was expected to receive either treatment or placebo for six months. Twelve people did not show up at the 12-week follow-up because they lived too far from the clinical unit and 8 stopped treatment due to the high number of pills taken each day. Luckily enough, as described in Table 1, the two residual groups completing the study turned out to be equally distributed and displayed no significant differences.
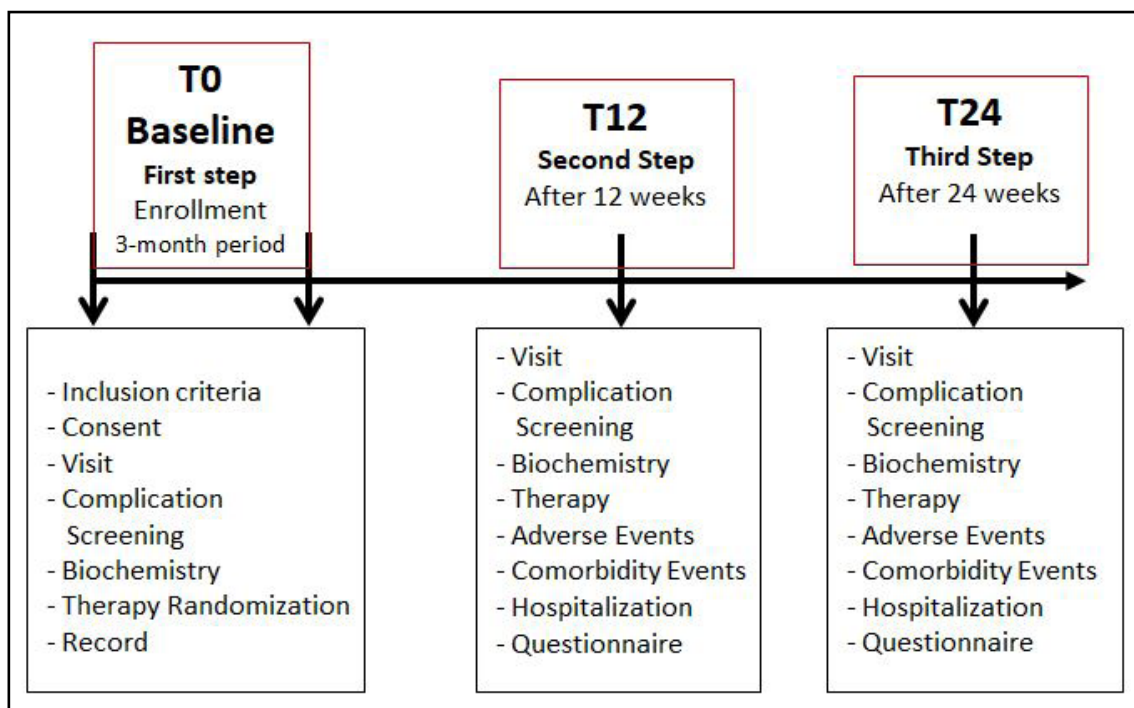

Figure 1: Schematic representation of the study protocol

	Treatment Group	Placebo Group	*p*
Subjects (n.)	50	50	-
Male (n.)	25	27	.315
Age (year)	53.03±7.11	54.16±6.14	.287
Waist circumference (cm)	115.00±9.12	113.41±13.22	.671
BMI (kg/m²)	34.46±4.24	35.34±2.07	.512
Trunk fat level (%)	44.34±5.17	44.04±6.23	.214
Visceral fat level (%)	24.05±6.41	22.18±8.60	.338
Systolic BP (mmHg)	148.42±12.68	146.39±11.07	.619.
Diastolic BP (mmHg)	98.32±5.75	97.48±5.67	.227
Heart rate (beats/min)	73.04±7.61	72.82±9.37	.189
Fasting plasma glucose (mg/dl) IFG sujects [n 7] **IGT subjects [n 47]**	114.73±2.81 95.60±3.13	115.06±2.84 93.40±3.53	.214 .324
2h after a 75-g OGTT (mg/dl) IFG subjects [n 7] **IGT subjects [n 47]**	130.51±3.36 179.6±8.6	128.62±2.82 181.6±3.2	.314 .226
Fasting plasma glucose overall (mg/dl)	97.31±2.03	96.43±1.81	.307
HbA1c (%)	5.26±0.37	5.19±0.48	.229

	Treatment Group	Placebo Group	*p*
HOMA-R	4.80±0.82	4.54±0.75	.313
eGFR (ml/min/1,73 m²)	86.07±9.15	89.21±12.35	.417
Total cholesterol (mg/dl)	230.18±18.34	237.43±15.53	.289
HDL cholesterol (mg/dl)	41.65±3.49	41.37±4.19	.216
LDL cholesterol (mg/dl)	151.09±18.75	153.67±14.48	.331
Triglycerides (mg/dl)	198.37±23.62	199.28±18.32	.724
AST (IU/L)	29.16±9.02	29.24±8.20	.338
ALT (IU/L)	29.33±8.06	27.65±9.09	.187
γGT (IU/L)	21.32±7.23	24.33±5.27	.219
Uric Acid (mg/dl)	7.52±1.34	7.47±1.56	.611
Cardiovascular risk factors			
Elevated waist-hip ratio (%)	65	61	.229
Recent or current smokers (%)	15	16	.174
Low concentration of HDL cholesterol (%)	60	62	.310
Family history of premature heart disease (%)	10	11	.243
Hypertension (%)	58	55	.267
> 2 Risk Factors (%)	79	80	.335

Table 1: Clinically relevant parameters at baseline: no significant differences were found between groups. Absolute, percent or M ± SD values were reported depending on individual parameters. (*Treatment Group = on Kirocomplex; P Group = Placebo Group*)

During the study all patients received a low-calorie diet (20–25% less than the amount of calories required to maintain current weight) varying in percentages of proteins (10–20%), fat (20–30%, saturated being less than 10%), and carbohydrates (50–60%, sucrose being less than 5%) to meet individual tastes and wishes at best. Favorite carbohydrate sources were starches with a low glycemic index and high soluble fiber content (35 g/day). Enrolled patients did not exceed 3000 steps per day, i.e. 3 Metabolic Equivalent of Task / day (MET), and ingested less than 30 or 15 g ethanol per day whether male or female, respectively; 16 subjects from the treatment group and 14 from the control group were smokers (13 ± 4 cigarettes / day and 10 ± 5 / day, respectively).

The following parameters were measured at baseline, as well as, 12 and 24 weeks into the study by a high performance autoanalyzer (Automatic biochemistry analyzer with integrated system Selecta Pro XI, Elithech, USA): Fasting Blood Glucose, Insulin, Total Cholesterol, HDL Cholesterol, LDL Cholesterol, Triglycerides, and Uric Acid. HOMA-R (i.e. Insulin Resistance index) was calculated according to the Homeostatic Model Assessment). Anthropometry (weight, height, BMI, waist circumference or WC, waist to hip ratio) and bioimpedance results (percent abdominal fat) were also recorded at the same time points.

Safety Parameters

Besides AST, ALT, total bilirubin, serum albumin, γ-glutamyltransferase, alkaline phosphatase, urea nitrogen and creatinine measured through the above mentioned autoanalyzer, red/white blood cell and platelet counts (Countess II Automated Cell Counter, Thermo Fischer, USA) were recorded at baseline and after 3 and 6 months along with any side effects assessed using a semi-quantitative scale.

Bioimpedance Analysis (BIA): Abdominal fat was measured using an innovative bioimpedance device (Bia-TANITA AB140 ViScan, BIO) [37], consisting of a laser beam guided band pointing to the navel with four electrodes placed directly on the abdomen, being the subject in the supine position. To ensure highly accurate and repeatable results, waist circumference (WC) was measured using the base unit itself with an infrared system. WC, trunk fat (TF), and percent visceral fat (VF) were thus evaluated at enrollment and after 6 and 12 months of treatment.

Diet Adherence: All subjects completed a questionnaire (Q) to evaluate diet adherence during the treatment period. Q consisted of five questions including the frequency of nutritional advice breaches and the type and amount of food eaten outside the recommended value. Four deviations per week from the recommended diet within 250 calories The questionnaire had been previously tested for validity and reliability in a sample of 10 health care workers, by verifying the concordance of the answers given three times in a two-week period by the same subject (mean concordance degree of 95±5 %) as previously described [38].

Cardiovascular Event Risk Score: The 10 year chance to undergo cardiovascular events was calculated on the basis of the CV risk score developed by the Italian Health Institute (ISS, Istituto Superiore di Sanità; "Progetto Cuore") taking into account age, gender, smoking habits, blood pressure levels, glucose, total and HDL cholesterol [39-41].

Statistics: On the basis of the primary outcome endpoint, the estimated sample size for this study to have a power of 90% with an alpha error of 0.02 to yield a statistically significant result was 50 pairs of subjects. After taking into consideration the presence of a secondary outcome and the possibility of dropouts, we decided to increase our sample size, including all 60 consecutive pairs of

subjects who met the inclusion criteria. Results were expressed as mean ± SD or % of the fifty couples who completed the study. Ten couples dropped out for personal reasons, not because of any side effects. Observed treatment and differences were tested by repeated measures analysis of variance (rANOVA) integrated by the Bonferroni test and the two-tailed paired Student's t-test with 95% Confidence Intervals (CI) for parametric and Mann–Whitney's U test for non-parametric variables defined according to the Kolmogorov-Smirnov test. The χ^2 test with Yates correction or Fisher Exact test was used to compare categorical variables. A p<0.05 was chosen as the least acceptable level of statistical significance. All the evaluations were performed using the SPSS/PC+ software (IBM SPSS Statistics version 18.2).

Results

Adherence to diet was high in both groups (89% vs 92% in K and P, respectively; p n.s.).

Reported side effects were mild and quite similar across groups. In greater detail, the following were recorded in K vs P subjects, respectively: drowsiness (n=1 vs n=1), acid regurgitation (n=2 vs n=2), post-prandial nausea/vomiting (n=1 vs n=1), itching (n=1 vs n=1), headache (n=1 vs n=3), dizziness and/or fainting (n=1 vs n=1), cold sweat with/without hunger pangs (n=2 vs n=3), palpitation (n=2 vs n=3), tachycardia (n=1 vs n=1). No people experienced constipation. In most cases unwanted effects tended to resolve spontaneously during treatment. Percentage variations in biochemical safety parameters during the study period were similar in the two groups, ranging 0.2 to 0.5%.

As summarized in Table 2 and 3, most investigated parameters significantly and steadily improved in the T Group with respect to both baseline and P Group. In greater detail, active treatment proved to be able to significantly reduce uric acid, systolic/diastolic blood pressure levels in the T group, and to cause a more relevant decrease in the latter than in the P Group as for BMI, WC (mean difference 27±3 cm vs 13±3 cm, respectively; p<0.01), as well as, in percent TF (13.5±2% vs 8±1%, respectively) and VF (10±1% vs 3±1%, respectively) (Figures 2 and 3). Other modifiable cardiovascular risk factors also displayed the following % improvement with respect to baseline: waist to hip ratio -12.7% vs -5.2% (p<0.001); HDL cholesterol +17.2% vs +2.4% (p<0.001); systolic BP -8.2% vs -2.1% (p<0.01); diastolic BP -5.9% vs -1.5% (p<0,01) in the T versus the P group, respectively. HbA1c levels kept within normal limits and displayed virtually no changes while fasting plasma glucose levels significantly decreased in the T group only, as summarized in Table II bis,.

Comparison of lipid parameters between treatment groups							
	Total Cholesterol (mg/dl)		HDL Cholesterol Cholesterol (mg/dl)		Triglycerides (mg/dl)		LDL Cholesterol (mg/dl)
	T Group	P Group	T Group	P Group	T Group	P Group	T Group	P Group
Baseline T0	230.18±18.34	237.43±15.53	41.65±3.49	41.37±4.19	198.37±23.62	199.28±18.32	151.09±18.75	153.67±14.48
12 weeks % Δ vs T0	201.44±14.43* -13**	217.76±18.97 -8	43.65±4.67 +5	42.41±4,34 +3	176.69±16.74* -11*	187.41±16.65-6	123.35±12.43* -18*	138.68±15.74 -10
24 weeks % Δ vs T0	178.43±16.19**# -22**	198.58±17.81* -16*	48.95±3.76*# +18*	42.49±4.33 +3	148.35±13.45**# -25**	185.57±15.68-7	100.48±14.39**# -33**	139.49±12.70 -9

***p<0.01, *p<0.05 vs baseline; # p<0.01, § p<0.05 vs Placebo group*

Comparison of HOMA-R and bioimpedance parameters between treatment groups							
	HOMA-R		WC (cm)		TF (%)		VF (%)
	T Group	P Group	T Group	P Group	T Group	P Group	T Group	P Group
Baseline T0	4.81±0.82	4.55±0.74	115.00±9.12	113.41±13.22	44.34±5.17	44.04±6.23	24.05±6.41	22.18±8.60
12 weeks % Δ vs T0	3.3±0.8* -31*	4.43±0.62 - 2	96.22±4.17* -17	111.43±11.30 -2	36.14±4.02* -17*	40.06±6 -9	19.06±6.24* -21*	20.41±8.25 -8
24 weeks % Δ vs T0	2.63±0.51**# - 46**	4.34±0.61 -4	88.24±5.31*# - 23*	100.57±!4.34 -11	31.46±3.24**# -29**	39.98±5.89 -9	14.43±4.24** # -40**	19.97±9.97 -10

***p<0.01, *p<0.05 vs baseline; # p<0.01, § p<0.05 vs Placebo group*

	Uric Acid (mg/dl)		SBP (mmHg)		DBP (mmHg)		HbA1c (%)
	T Group	P Group	T Group	P Group	T Group	P Group	T Group	P Group
Baseline T0	7.52±1.34	7.47±1.56	148.42±12.68	146.39±11.07	98.32±5.75	97.48±5.67	5.26±0.37	5.19±0.48
12 weeks % Δ vs T0	5.45±0.63**# -28**	7.28±0.98 -3	126.67±18.86*§ -15	141.87±11.92 -3	81.57±4.91* -17	96.58±6.76 -1	5.26±0.44 0	5.19±0.46 0
24 weeks % Δ vs T0	4.29±0.58**# -43**	7.09±0.57* -5	123.86±15.57**# -17	138.76±14.86**# -5	78.57±8.44**# -18	95.38±9.86 -2	5.45±0.37 +4	5.47±0.28 +5

***p<0.01, *p<0.05 vs baseline; # p<0.01, § p<0.05 vs Placebo group*

Table 2: Effects of a 24-week treatment on biochemical, clinical and bioimpedance parameters (M±SD); (*T Group and P Group as reported in Table 1*)

FASTING PLASMA GLUCOSE (mg/dl)			
FASTING PLASMA GLUCOSE	FASTING PLAS-MA GLUCOSE	FASTING PLASMA GLUCOSE	FASTING PLAS-MA GLUCOSE
IFG patients (n = 7) (mg/dl) Baseline	114.72±2.80	115.81±2.83	.6281
24 weeks % Δ vs T0	81.16±3.21* (-29%)	104.13±3.41 (-10%)	.0365
IGT patients (n = 47) (mg/dl) Baseline	95.62±3.13	93.42±3.53	.5837
24 weeks % Δ vs T0	80.25±3.36 * (-16%)	84.07±4.16 (-10%)	.8292
Overall (n = 50) (mg/dl) Baseline	97.31±2.03	96.43±1.81	.6374
24 weeks % Δ vs T0	80.33±4.31 (-19%)	87.24±4.25 (-10%)	.559
* p vs baseline			

Table 3: Fasting plasma glucose levels observed in IGF and IGT patients
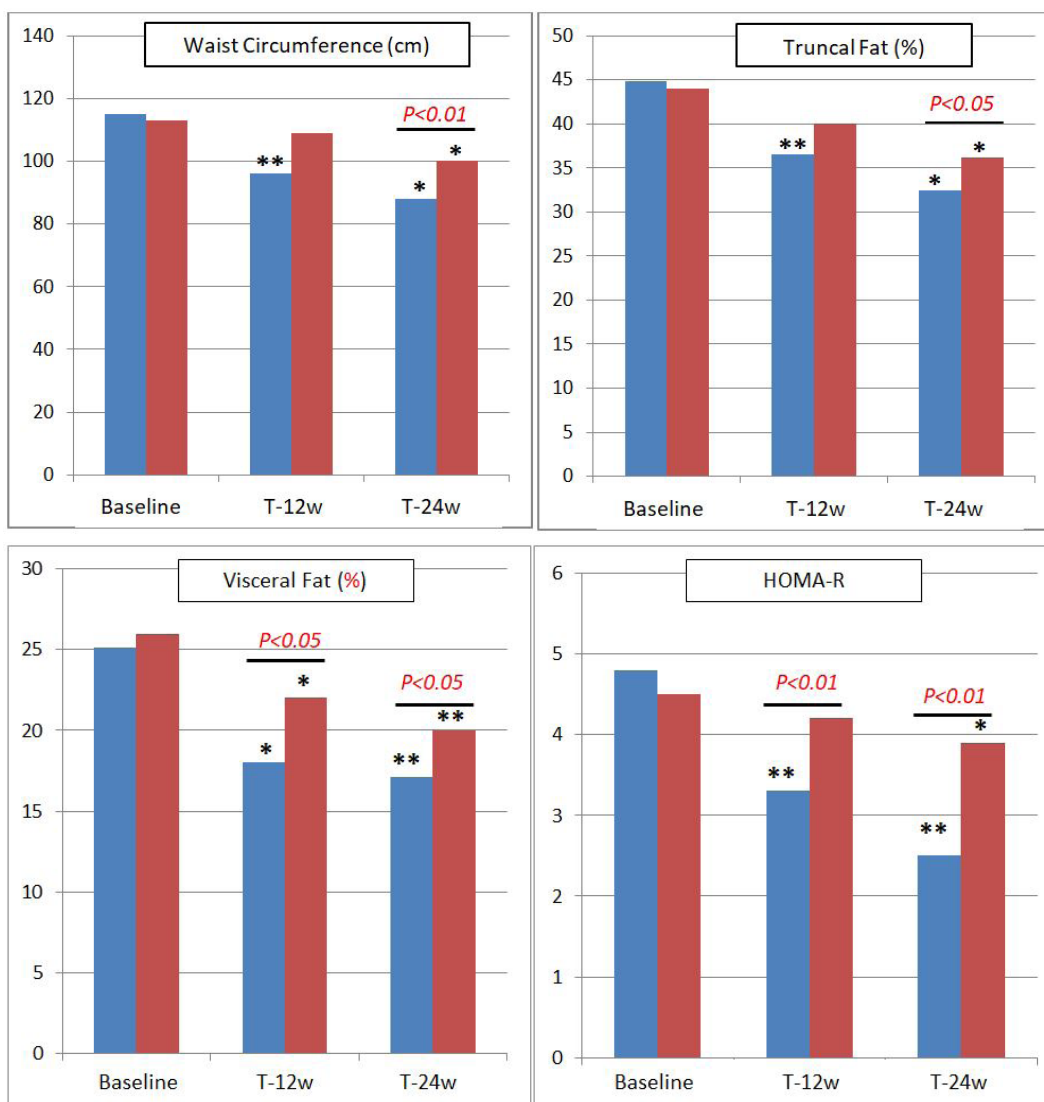
Figure 2: Comparison between Treatment Group (blue columns) and Placebo Group (red columns) with respect to Waist Circumference (cm), Truncal Fat (%), and Visceral Fat (%) at 12 (T12-w) and 24 (T24-w) treatment-week: * $p<0.05$ vs baseline; **$p<0.01$ vs baseline: the horizontal bars indicate the significance of the differences between groups at the single time points
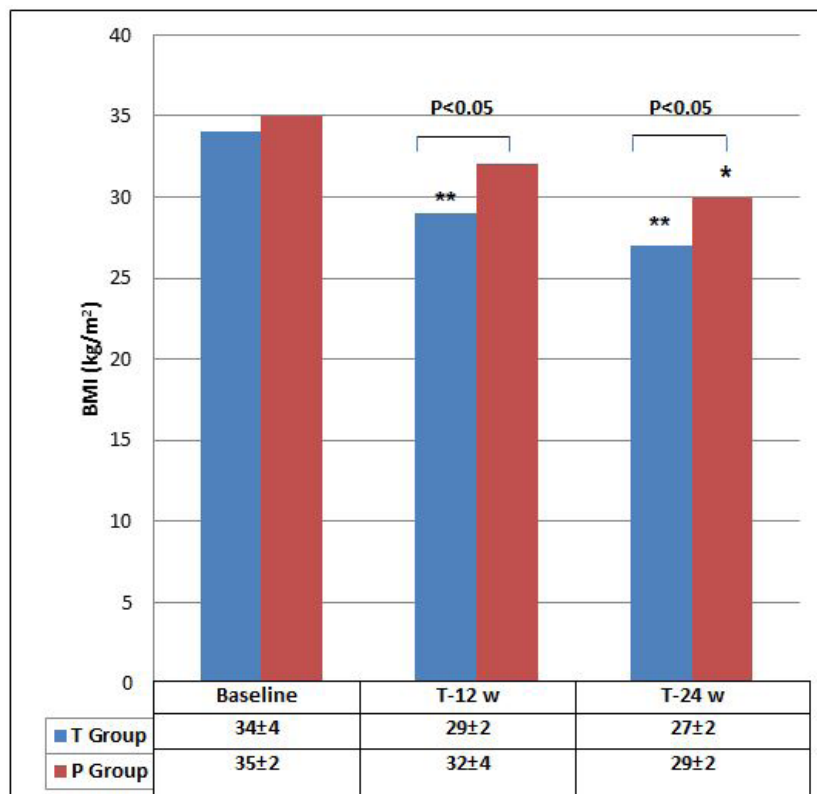
Figure 3: Comparison between Treatment Group (blue columns) and Placebo Group (red columns) with respect to BMI (M±DS; kg/m²) during a 24-week treatment period (**<0.01 vs baseline; * p<0.05 vs baseline)

Baseline CV event risk scores were statistically undistinguishable between groups and were therefore pooled together for further analysis by gender and smoking habits, which showed mean values of 12.1±1.6 (range 8.4-14.7) in males, with a marked difference between nonsmokers (10.1±1.2, range 7.8-11.8) and smokers (14.6±2.2, range 10.5-16.9), and of 10.1±2.1 (range 8.8-13.3) in females.

Discussion

The association of D-Chiro-Inositol, Myo-Inositol and Polygonum cuspidatum extract on Magnesium hydroxide titrated to at least 30% Resveratrol is a stable nutraceutical combination, marketed under the name of Kirocomplex®. The complementary biological characteristics of all three components have been proven by several *in vitro* and *in vivo* studies, and especially in clinical conditions characterized by high insulin-resistance as obesity, metabolic syndrome or gestational diabetes. Their beneficial health effects are essentially based on powerful anti-oxidant properties expected to act as preventative and therapeutic tools against chronic-degenerative diseases including atherosclerosis, metabolic syndrome and or obesity, osteoporosis, diabetes, cancer, some inflammatory and neuro-degenerative conditions, as well as, skin aging [21-30].

If the favorable effects of MY and DCI in various morbid conditions are well established, there are still some uncertainties concerning the clinical relevance of R. In fact, many *in vitro* and preclinical studies [42-44] point to its anti-inflammatory effect and suggest its use in cardio-vascular diseases, obesity, dyslipidemia and metabolic syndrome with and without overt diabetes mostly based on observed changes in risk factors for those diseases. In particular, in an *in vitro* model on Human Simpson-Golabi-Behmel syndrome pre-adipocytes and adipocytes, R inhibited preadipocyte proliferation and adipogenic differentiation in a Sirt1-dependent manner [42]. Moreover, in human adipocytes, it enhanced basal and insulin-stimulated glucose uptake, inhibited *de novo* lipogenesis by reducing lipogenic gene expression and down-regulated the expression and secretion of interleukin-6 and interleukin-8, thus proving to act on adipose tissue mass and function in such a way as to potentially counteract obesity and related comorbidities [42].

Preclinical trials [45,46] suggest that R mimics the metabolic effects of calorie restriction. In experimental animals, this potential translates into prevention or improvement of inflammation, disglycemia, cancer, and nonalcoholic fatty liver disease. A meta-analysis including 736 subjects from 17 RCTs concerning the effects of Resveratrol supplementation on inflammatory markers provided evidence in favor of a significant reduction of TNF-α and hs-CRP levels [44]. Nevertheless, evidences of positive effects in human diseases are scanty. The lipid profile improved in both groups, probably depending on diet effect *per se*, yet got significantly better in those on active treatment.

Our study addresses clinical objectives for the first time in a population at high risk for cardiovascular disease, documenting a series of beneficial effects of Reversatrol in association to MY and DCI.

No relevant side effects were recorded in the T group *per se* and compared to the P Group, as also witnessed by no treatment discontinuation.

The fact that most metabolic results were observed only in the T group can find explanations in the effects of individual tablet components. In fact, MY insulin resistance counteracting ability is well known from studies performed in women with the polycystic ovary syndrome, and R has been described as a calorie restriction mimicking agent and reported to improve exercise performance, energy expenditure and insulin sensitivity, as well as, to reduce body fat content through blunted adipogenesis and increased lipid mobilization from the adipose tissue [47].

The potential anti-obesity effects was supported by numerous cell-culture and animal studies showing that those compounds regulate energy intake / expenditure, adipocyte life cycle and function, white adipose tissue (WAT) inflammation, and gut microbiota composition by targeting multiple molecules and signaling pathways [48].

Improved uric acid levels in the treatment group deserves special consideration based on the fact that high uric acid levels are considered proinflammatory and have been extensively reported to be associated with cardiovascular risk score [49-54].

The favorable changes observed in HOMA index, lipid profile, and general parameters such as body composition and CV risk factors, including arterial pressure, can also be explained by the anti-inflammatory effects of the two associated compounds *per se* i.e. independently of fasting plasma glucose and HbA1c levels [55].

Conclusion

The results of our 24-week pilot study on 50 pairs of pre-diabetic subjects without previous CV-events and with obesity/overweight, high insulin resistance and altered lipid profile show that the nutraceutical association under study was able to improve (i) several metabolic parameters and insulin sensitivity, as witnessed by HOMA-R decrease, (ii) cardiovascular risk factors, such as blood pressure, lipid profile and urate levels [39], and (iii) anthropometric parameters, including body fat distribution. All this suggests its ability to positively affect energy balance at multiple sites with respect to placebo. These results are particularly relevant in the subjects studied, because they have a high cardio-vascular risk, due to overweight / obesity, dyslipidemia, marked insulin-resistance and high risk of developing diabetes.

Treatment seems to be safe and tolerated at the tested doses, with minimal unwanted effects, contrary to what sometimes attributed to resveratrol [43].

The originality of our findings lies on the following: (i) the study was conducted for 24 weeks on a homogeneous series of subjects with a high cardio-vascular risk; (ii) a significantly lower abdominal adiposity was attained in T Group; and (iii) for the first time a significant decreasing effect on circulating uric acid was described in T Group.

Our results warrant further confirmation in larger and longer trials, of course, but point to a new possible clinical use of Kirocomplex®, aimed at treating abdominal fat in overweight/obese patients with high cardio-vascular risk factors, and further pave the way to RCTs of primary and secondary prevention on patients with metabolic diseases well established with and without CV-events [56].

Limitations

The main limitations of our study are the small number of subjects and the limited period of observation. However, this choice reflects our will to test the association of myinositol, D-chiro-inositol and resveratrol on a limited number of subjects and for a short period first, before organizing a wider and longer-lasting trial. Another limitation is the large ranges of our population age and BMI. Nevertheless, the latter limitation is somewhat mitigated by the close similarity displayed by those two parameters between groups, which should in fact give credibility to our results.

Compliance with Ethical Standards

Ours was a spontaneously born, unconditioned study organized and authorized by the Ethics Committee of the Campania University "Luigi Vanvitelli", Naples, Italy ((Trial registration number 119, 10.01.2018).).

Ethical Standard

This study was conducted in conformance with good clinical practice standards. The study was led in accordance with the Declaration of Helsinki 1975, as revised in 2008.

Human and Animal Rights

All followed procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national).

Informed Consent

Written informed consent was obtained from all participants before enrollment.

Acknowledgment

References

1. Reaven GM (1988) Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37: 1595-607.

2. Wyne KL (2003) Free fatty acids and type 2 diabetes mellitus. Am J Med 115: 29S-36S.

3. Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL (1996) Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest 97: 2859-65.

4. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M (2001) Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J Clin Invest 108: 1341-8.

5. Berg AH, Scherer PE (2005) Adipose tissue, inflammation, and cardiovascular disease. Circ Res 96: 939-49.

6. Romero G, Luttrell L, Rogol A, Zeller K, Hewlett E, et al. (1988) Phosphatidylinositol-glycan anchors of membrane proteins: potential precursors of insulin mediators. Science 240: 509-11.

7. Sun TH, Heimark DB, Nguygen T, Nadler JL, Larner J (2002) Both myo-inositol to chiro-inositol epimerase activities and chiroinositol to myo-inositol ratios are decreased in tissues of GK type 2 diabetic rats compared to Wistar controls. Biochem Biophys Res Commun 293: 1092-8.

8. Field MC (1997) Is there evidence for phospho-oligosaccharides as insulin mediators? Glycobiology 7: 161-8.

9. Pintaudi B, Di Vieste G, Bonomo M (2016) The Effectiveness of Myo-Inositol and D-Chiro Inositol Treatment in Type 2 Diabetes. Int J Endocrinol 2016: 9132052.

10. Carlomagno G, Nordio M, Chiu TT, Unfer V (2011) Contribution of myo-inositol and melatonin to human reproduction. Eur J Obstet Gynecol Reprod Biol 159: 267-72.

11. Unfer V, Carlomagno G, Dante G, Facchinetti F (2012) Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. Gynecol Endocrinol 28: 509-15.

12. Saleem F, Rizvi SW (2017) New Therapeutic Approaches in Obesity and Metabolic Syndrome Associated with Polycystic Ovary Syndrome. Cureus 9: e1844.

13. Santamaria A, Di Benedetto A, Petrella E, Pintaudi B, Corrado F, et al. (2016) Myo-inositol may prevent gestational diabetes onset in overweight women: a randomized, controlled trial. J Matern Fetal Neonatal Med 29: 3234-7.

14. D'Anna R, Di Benedetto A, Scilipoti A, Santamaria A, Interdonato ML, et al. (2015) Myo-inositol supplementation for prevention of gestational diabetes in obese pregnant women: a randomized controlled trial. Obstet Gynecol 126: 310-5.

15. Crawford TJ, Crowther CA, Alsweiler J, Brown J (2015) Antenatal dietary supplementation with myo-inositol in women during pregnancy for preventing gestational diabetes. Cochrane Database of Syst Rev 12: CD0115077.

16. D'Anna R, Scilipoti A, Giordano D, Caruso C, Cannata ML, et al. (2013) Myo-inositol supplementation and onset of gestational diabetes mellitus in pregnant women with a family history of type 2 diabetes: a prospective, randomized, placebo-controlled study. Diabetes Care 36: 854-7.

17. Corrado F, D'Anna R, di Vieste G, Giordano D, Pintaudi B, et al. (2011) The effect of myoinositol supplementation on insulin resistance in patients with gestational diabetes. Diab Medicine 28: 972-5.

18. Lubin V, Shojai R, Darmon P, Cosson E (2016) A pilot study of gestational diabetes mellitus not controlled by diet alone: first-line medical treatment with myo-inositol may limit the need for insulin. Diabetes Metab 42: 192-5.

19. Mancini M, Andreassi A, Salvioni M, Pelliccione F, Mantellassi G, et al. (2016) Myoinositol and D-Chiro Inositol in Improving Insulin Resistance in Obese Male Children: Preliminary Data. Int J Endocrinol 2016: 8720342.

20. Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 5: 493-506.

21. De Amicis F, Giordano F, Vivacqua A, Pellegrino M, Panno ML, et al. (2011) Resveratrol, through NF-Y/p53/Sin3/HDAC1 complex phosphorylation, inhibits estrogen receptor α gene expression via p38MAPK/CK2 signaling in human breast cancer cells. FASEB J 25: 3695-707.

22. Ma X, Sun Z, Liu Y, Jia Y, Zhang B, et al. (2013) Resveratrol improves cognition and reduces oxidative stress in rats with vascular dementia. Neural Regen Res 8: 2050-9.

23. Cao C, Lu R, Kivlin B, Wallin E, Card E, et al. (2009) SIRT1 confers protection against UVB- and H2O2-induced cell death via modulation of p53 and JNK in cultured skin keratinocytes J Cell Mol Med 13(9B): 3632-43.

24 Nichols JA, Katiyar SK (2010) Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. Arch Dermatol Res 302: 71-83.

25. Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, et al. (2002) Mechanisms of photoaging and chronological skin aging. Arch Dermatol 138: 1462-70.

26. Stojanović S, Sprinz H, Brede O (2001) Efficiency and mechanism of the antioxidant action of trans-resveratrol and its analogues in the radical liposome oxidation. Arch Biochem Biophys 391: 79-89.

27. Yan F, Sun X, Xu C (2018) Protective effects of resveratrol improve cardiovascular function in rats with diabetes. Exp Ther Med 15: 1728-34.

28. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, et al. (2004) Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res 24: 2783-840.

29. Walle T, Hsieh F, DeLegge MH, Oatis JE Jr, Walle UK (2004) High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab Dispos 32: 1377-82.

30. Howells LM, Berry DP, Elliott PJ, Jacobson EW, Hoffmann E, et al. (2011) Phase I randomized double-blind pilot study of micronized resveratrol (SRT501)In patients with hepatic metastases-safety, pharmacokinetics and pharmacodynamics. Cancer Prev Res 4: 1419-25.

31. Chan WK, Delucchi AB (2000) Resveratrol, a red wine constituent, is a mechanism-based inactivator of cytochrome P450 3A4. Life Sci 67: 3103-12.

32. Amri A, Chaumeil JC, Sfar S, Charrueau C (2012) Administration of resveratrol: what formulation solutions to bioavailability limitations? J Control Release 158: 182-93.

33. Malvasi A, Kosmas I, Mynbaev OA, Sparic R, Gustapane S, et al. (2017) Can trans resveratrol plus d-chiro-inositol and myo-inositol improve maternal metabolic profile in overweight pregnant patients? Clin Ter 168: e240-7.

34. Green S (1991) How many subjects does it take to do a regression analysis. Multivar Behav Res 26: 499-510.

35. WHO (2019) Obesity and overweight. WHO definition, Switzerland.

36. Professional Practice Committee: Standards of Medical Care in Diabetes-2018. Diabetes Care 41(Supplement 1): S3.

37. Vitturi N, Soattin M, De Stefano F, Vianello D, Zambon A, et al. (2015) Ultrasound, anthropometry and bioimpedance: a comparison in predicting fat deposition in non-alcoholic fatty liver disease. Eat Weight Disord 20: 241-7.

38. Guarino G, Ragozzino G, Della Corte T, Fontana S, Strollo F, et al. (2018) Selenium Supplementation in Obese Patients with Subclinical Hypothyroidism and Type 2 Diabetes. J Nutr Health Sci 5: 202.

39. Giampaoli S, Palmieri L, Donfrancesco C, Lo Noce C, Pilotto L, et al. (2015) Cardiovascular health in Italy. Ten-year surveillance of cardiovascular diseases and risk factors: Osservatorio Epidemiologico Cardiovascolare/Health Examination Survey 1998-2012. Eur J Prev Cardiol 22(2 Suppl): 9-37.

40. Giampaoli S, Palmieri L, Panico S, Vanuzzo D, Ferrario M, et al. (2006)Favorable cardiovascular risk profile (Low Risk) and 10-year stroke incidence in women and men: Findings on 12 Italian population samples. Am J Epidemiol 163: 893-902.

41. Palmieri L, Donfrancesco C, Giampaoli S, Trojani M, Panico S, et al. (2006) Favorable cardiovascular risk profile and 10-year coronary heart disease incidence in women and men: Results from the Progetto CUORE. Eur J Cardiovasc Prev Rehabil 13: 562-70.

42. Fischer-Posovszky P, Kukulus V, Tews D, Unterkircher T, Debatin KM, et al. (2010) Resveratrol regulates human adipocyte number and function in a Sirt1-dependent manner. Am J Clin Nutr 92: 5-15.

43. Vang O, Ahmad N, Baile CA, Baur JA, Brown K, et al. (2011) What is new for an old molecule? Systematic review and recommendations on the use of resveratrol. PLoS One 6: e19881.

44. Patel KR, Scott E, Brown VA, Gescher AJ, Steward WP, et al. (2011) Clinical trials of resveratrol. Ann N Y Acad Sci 1215: 161-9.

45. Poulsen MM, Jørgensen JO, Jessen N, Richelsen B, Pedersen SB (2013) Resveratrol in metabolic health: an overview of the current evidence and perspectives. Ann N Y Acad Sci 1290: 74-82.

46. Tomé-Carneiro J, Gonzálvez M, Larrosa M, Yáñez-Gascón MJ, García-Almagro FJ, et al. (2012) One-year consumption of a grape nutraceutical containing resveratrol im'proves the inflammatory and fibrinolytic status of patients in primary prevention of cardiovascular disease. Am J Cardiol 110: 356-63.

47. pringer M, Moco S (2019) Resveratrol and Its Human Metabolites-Effects on Metabolic Health and Obesity. Nutrients 11: E143.

48. Pan MH, Wu JC, Ho CT, Lai CS (2018) Antiobesity molecular mechanisms of action: Resveratrol and pterostilbene. Biofactors 44: 50-60.

49. Borghi C, Rodriguez-Artalejo F, De Backer G, Dallongeville J, Medina J, et al. (2018) Serum uric acid levels are associated with cardiovascular risk score: A post hoc analysis of the EURIKA study. Int J Cardiol 253: 167-73.

50. Carluccio E, Coiro S, Ambrosio G (2018) Unraveling the relationship between serum uric acid levels and cardiovascular risk. Int J Cardiol 253:174-5.

51. Zheng BK, Li N (2018) Association of serum uric acid levels and cardiovascular risk score. Int J Cardiol 258: 297.

52. Sun GZ, Wang HY, Chen YT, Sun YX (2018) Serum uric acid levels positively correlates with 10-year cardiovascular risk score in the general population from China. Int J Cardiol 266: 259.

53. Borghi C (2018) Gout and CV disease: It is time to move on. Int J Cardiol 270: 311.

54. Calvo Aranda E, Andres M, Gonzalez Martin J, Abdelkader Abu-Sneimeh A, Carrion O, et al. (2018) Urate crystals and inflammation. Cardiovascular impact of gout. Int J Cardiol 271: 295.

55. Mazidi M, Katsiki N, Mikhailidis DP, Banach M (2019) Association of ideal cardiovascular health metrics with serum uric acid, inflammation and atherogenic index of plasma: A population-based survey. Atherosclerosis 284: 44-9.

56. Koushki M, Dashatan NA, Meshkani R (2018) Effect of Resveratrol Supplementation on Inflammatory Markers: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Clin Ther 40: 1180-92.