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

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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 deacetylase (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 lipophilicity-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;
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.

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

Figure 1: Schematic representation of the study protocol
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment Group</th>
<th>Placebo Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-R</td>
<td>4.80±0.82</td>
<td>4.54±0.75</td>
<td>.313</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>86.07±9.15</td>
<td>89.21±12.35</td>
<td>.417</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>230.18±18.34</td>
<td>237.43±15.53</td>
<td>.289</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>41.65±3.49</td>
<td>41.37±4.19</td>
<td>.216</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>151.09±18.75</td>
<td>153.67±14.48</td>
<td>.331</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>198.37±23.62</td>
<td>199.28±18.32</td>
<td>.724</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>29.16±9.02</td>
<td>29.24±8.20</td>
<td>.338</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>29.33±8.06</td>
<td>27.65±9.09</td>
<td>.187</td>
</tr>
<tr>
<td>γGT (IU/L)</td>
<td>21.32±7.23</td>
<td>24.33±5.27</td>
<td>.219</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>7.52±1.34</td>
<td>7.47±1.56</td>
<td>.611</td>
</tr>
</tbody>
</table>

**Cardiovascular risk factors**

- Elevated waist-hip ratio (%): 65 vs. 61, p = .229
- Recent or current smokers (%): 15 vs. 16, p = .174
- Low concentration of HDL cholesterol (%): 60 vs. 62, p = .310
- Family history of premature heart disease (%): 10 vs. 11, p = .243
- Hypertension (%): 58 vs. 55, p = .267
- > 2 Risk Factors (%): 79 vs. 80, p = .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 Fisher, 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 χ² 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=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.

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

**Comparison of lipid parameters between treatment groups**

<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>LDL Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T Group</td>
<td>P Group</td>
<td>T Group</td>
<td>P Group</td>
</tr>
<tr>
<td>Baseline T0</td>
<td>230.18±18.34</td>
<td>237.43±15.53</td>
<td>41.65±3.49</td>
<td>41.37±4.19</td>
</tr>
<tr>
<td>12 weeks % Δ vs T0</td>
<td>201.44±14.43*</td>
<td>217.76±18.97</td>
<td>43.65±4.67</td>
<td>42.41±4.34</td>
</tr>
<tr>
<td>24 weeks % Δ vs T0</td>
<td>178.43±16.19** #</td>
<td>198.58±17.81*</td>
<td>48.95±3.76* #</td>
<td>42.49±4.33 #</td>
</tr>
</tbody>
</table>

**Comparison of HOMA-R and bioimpedance parameters between treatment groups**

<table>
<thead>
<tr>
<th></th>
<th>HOMA-R</th>
<th>WC (cm)</th>
<th>TF (%)</th>
<th>VF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T Group</td>
<td>P Group</td>
<td>T Group</td>
<td>P Group</td>
</tr>
<tr>
<td>Baseline T0</td>
<td>4.81±0.82</td>
<td>4.55±0.74</td>
<td>115.00±9.12</td>
<td>113.41±13.22</td>
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<tr>
<td>12 weeks % Δ vs T0</td>
<td>3.3±0.9** #</td>
<td>4.3±0.62</td>
<td>96.22±4.17*</td>
<td>111.43±11.30</td>
</tr>
<tr>
<td>24 weeks % Δ vs T0</td>
<td>2.63±0.51** #</td>
<td>8.84±5.31** #</td>
<td>100.57±14.34</td>
<td>31.46±3.24** #</td>
</tr>
</tbody>
</table>

**Comparison of biochemical, clinical and bioimpedance parameters (M±SD); (T Group and P Group as reported in Table 1)**

<table>
<thead>
<tr>
<th></th>
<th>Uric Acid (mg/dl)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>Hba1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T Group</td>
<td>P Group</td>
<td>T Group</td>
<td>P Group</td>
</tr>
<tr>
<td>Baseline T0</td>
<td>7.52±1.34</td>
<td>7.47±1.56</td>
<td>148.42±12.68</td>
<td>146.39±11.07</td>
</tr>
<tr>
<td>12 weeks % Δ vs T0</td>
<td>5.45±6.33** #</td>
<td>7.28±0.98</td>
<td>126.67±8.18* #</td>
<td>141.87±11.92</td>
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<tr>
<td>24 weeks % Δ vs T0</td>
<td>4.29±0.58** #</td>
<td>7.09±0.57*</td>
<td>123.86±15.57*</td>
<td>138.76±14.86*</td>
</tr>
</tbody>
</table>

**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)
**Table 3:** Fasting plasma glucose levels observed in IFG and IGT patients

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>24 weeks</th>
<th>% ∆ vs T0</th>
<th>24 weeks</th>
<th>% ∆ vs T0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IFG patients (n = 7)</strong> (mg/dl)</td>
<td>114.72±2.80</td>
<td>81.16±3.21*</td>
<td>(-29%)</td>
<td>104.13±3.41</td>
<td>(-10%)</td>
</tr>
<tr>
<td><strong>IGT patients (n = 47)</strong> (mg/dl)</td>
<td>95.62±3.13</td>
<td>80.25±3.36 *</td>
<td>(-16%)</td>
<td>93.42±3.53</td>
<td>(-10%)</td>
</tr>
<tr>
<td><strong>Overall (n = 50)</strong> (mg/dl)</td>
<td>97.31±2.03</td>
<td>80.33±4.31</td>
<td>(-19%)</td>
<td>96.43±1.81</td>
<td>(-10%)</td>
</tr>
</tbody>
</table>

* p vs baseline

**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
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, dysglycemia, 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.

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, dysglycemia, 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 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.
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