Effect of Ursolic Acid on Metabolic Syndrome, Insulin Sensitivity, and Inflammation

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ABSTRACT To evaluate the effect of ursolic acid on metabolic syndrome, insulin sensitivity, and inflammation. A randomized, double-blind, placebo-controlled clinical trial was carried out in 24 patients (30–60 years) with a diagnosis of metabolic syndrome without treatment. They were randomly assigned to two groups of 12 patients, each to receive orally 150 mg of ursolic acid or homologated placebo once a day for 12 weeks. Before and after the intervention, the components of metabolic syndrome, insulin sensitivity (Matsuda index), and inflammation profile (interleukin-6 and C-reactive protein) were evaluated. After ursolic acid administration, the remission of metabolic syndrome occurred in 50% of patients (P = .005) with significant differences in body weight (75.7 – 11.5 vs. 71 – 11 kg, P = .002), body mass index (BMI) (29.9 ± 3.6 vs. 24.9 ± 1.2 kg/m², P = .049), waist circumference (93 ± 8.9 vs. 83 ± 8.6 cm, P = .008), fasting glucose (6.0 ± 0.5 vs. 4.7 ± 0.4 mmol/L, P = .002), and insulin sensitivity (3.1 ± 1.1 vs. 4.2 ± 1.2, P = .003). Ursolic acid administration leads to transient remission of metabolic syndrome, reducing body weight, BMI, waist circumference and fasting glucose, as well as increasing insulin sensitivity.

INTRODUCTION

Metabolic syndrome refers to a set of pathological elements, including abdominal obesity, impaired glucose, abnormal lipid metabolism, and an elevated blood pressure, which increases the risk of developing cardiovascular diseases (CVDs) or type 2 diabetes mellitus (T2DM). This syndrome is characterized by a decreased insulin sensitivity, defined as the reduced ability of insulin to exert its biological effects during the regulation of glucose metabolism, that is to say, there is a decline in tissue response to normal levels or even high levels of circulating insulin, which is known as insulin resistance. The syndrome also occurs with a chronic inflammatory state at the systemic level, because of abnormal secretion of cytokines and other proinflammatory factors, which seems to start in the adipose tissue and continues with inflammatory changes in different organs.

Metabolic syndrome represents a great public health problem. The prevalence of metabolic syndrome in the world is about 25%, depending on the population studied and the diagnostic criteria used. Therefore, it becomes necessary to find an integral treatment that manages to encompass the greater number of components. In this sense, ursolic acid represents a great potential to achieve this purpose.

Ursolic acid is a pentacyclic triterpene carboxylic acid present as free acid or aglycone as part of saponins. It was considered inactive; however, in recent years a great interest has woken up because of its varied effects including anti-inflammatory, antioxidant, anticarcinogenic, antimicrobial, antiviral, and hepatoprotective actions. Interestingly, ursolic acid has also shown biological activities in both lipid and glucose metabolisms. The mechanisms by which it carries out these effects are multiple, and mainly related to actions on the insulin receptor, GLUT4, α-amylase, and glucotoxicity, as well as pancreatic lipase, adipogenesis, and lipolysis.

In this regard, the therapeutic potential of ursolic acid in the treatment of metabolic syndrome is evident. However, there is insufficient evidence from clinical trials that assess its effects. The aim of this study was to evaluate the effect of ursolic acid administration on metabolic syndrome, insulin sensitivity and inflammation.

MATERIALS AND METHODS

Design

Randomized, double-blind, placebo-controlled clinical trial.

Selection criteria. Patients of both sexes between 30 and 60 years of age, with diagnosis of metabolic syndrome.
according to the International Diabetes Federation (IDF) criteria\(^2\) (waist circumference \(\geq 90\) cm in men and \(\geq 80\) cm in women, in addition to two of the following criteria: fasting plasma glucose \(\geq 5.6\) mmol/L, triglycerides \(\geq 1.7\) mmol/L, high-density lipoprotein cholesterol [HDL-c] \(< 1.03\) mmol/L in men and \(< 1.29\) mmol/L in women, and blood pressure \(\geq 130/85\) mmHg) without pharmacological treatment were included. Patients with obesity grade II (body mass index [BMI] \(\geq 35\) kg/m\(^2\)), T2DM (fasting plasma glucose \(\geq 7\) mmol/L or postprandial blood glucose \(\geq 11.1\) mmol/L), dyslipidemia (triglycerides \(\geq 4.5\) mmol/L, total cholesterol \(\geq 6.2\) mmol/L, and/or LDL-c \(\geq 4.9\) mmol/L), or hypertension (\(\geq 140/90\) mmHg) were excluded.

Subjects. Subjects were selected from the same residential area and socioeconomic status. No participant was excessively sedentary (without programmed physical activity) or participated in heavy physical activity (>150 min/week of programmed physical activity). All individuals were nonsmokers and had a stable body weight for at least 3 months before the study. There was no personal history of hepatic, renal, or CVD. They had not consumed any medications known to affect any component of metabolic syndrome during the previous 6 months. Pregnancy, breastfeeding, or allergy to any component of the intervention were considered for exclusion criteria.

Pharmacological administration. After randomization, 12 patients received 150 mg of ursolic acid per day before breakfast for 12 weeks. The remaining 12 patients received calcined magnesia as placebo at the same dose. Interventions were encapsulated to achieve the same external appearance. All patients received general recommendations about their medical and nutritional therapy and were instructed to not modify their usual exercise routine.

End points. Metabolic syndrome components and insulin sensitivity were the primary end points. Transient remission of metabolic syndrome was evaluated, and it was considered when individuals no longer met the diagnostic criteria because of the improvement in some component of the syndrome. Anthropometric and inflammation measurements were secondary end points.

Procedures

Patients were evaluated before and after 12 weeks of the intervention. Tests were performed at 8:00 am after a 10- to 12-h overnight fast.

Clinical determinations were performed with the individuals wearing light clothing and after a resting period. Height was measured using a stadiometer with individuals standing without shoes, and the measurements were rounded off to the nearest centimeter. Body weight was measured with a bioimpedance digital scale (TBF-215 Body Composition Analyzer\(^5\); Tanita Corporation Itabashi, Tokyo, Japan) and the measurements were reported in kilograms with two decimals. BMI was calculated as weight divided by height\(^2\) and expressed as kg/m\(^2\). Waist circumference was measured with a flexible tape (Executive Thinline Tape Measure\(^6\); Lufkin Industries Sparks, Maryland, USA) at the midline between the highest point of the iliac crest and the lowest rib in the midaxillary line. Blood pressure was evaluated after a 15-min resting period with the individual sitting and determined using a digital sphygmomanometer (BPM-100\(^1\); BpTRU Medical Devices, Coquitlam, British Columbia, Canada), considering the mean of the three measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP), expressed in millimeters of mercury.

Biochemical determinations were performed after 3 days of an isocaloric diet (containing a minimum of 250 g of carbohydrates), women were tested during the first phase of their menstrual cycle (days 3–8). Venous blood samples were obtained and centrifuged at 700 × g using a calibrated centrifuge (Allegra\(^5\) 22R Centrifuge; Beckman Coulter Inc.; Brea, CA). Serum was separated into aliquots to perform laboratory analyses. Glucose and insulin were determined at baseline and 30, 60, 90, and 120 min after 75 g oral dextrose load in an oral glucose tolerance test (OGTT); the others were determined only from the baseline minute.

Glucose, triglycerides, total cholesterol, and HDL-c concentrations were determined by enzymatic colorimetric methods using an automated analyzer (XL-100\(^7\); Erba Diagnostics Mannheim GmbH Mannheim, Germany), with intra- and interassay coefficients of variation <2% for all measurements. Insulin concentrations were measured using the enzyme-linked immunosorbent assay (ELISA), which is based on the antigen–antibody reaction, using ELISA kits (Diagnostic Automation/ Ctz Diagnostics, Inc.; Calabasas, California, USA).

Insulin sensitivity was calculated using Matsuda index\(^1\) \((10,000/\sqrt{\text{glucose} \times \text{insulin}})/(\text{mean glucose OGTT} \times \text{mean insulin OGTT})\).

IL-6 and CRP concentrations were measured using ELISA technique (PeproTech EC Ltd., Rocky Hill, New Jersey, USA), with intra- and interassay coefficients of variation <5%.

Treatment adherence was monitored using a self-report diary as well as by recording of medication dispensed and returned by patients in every visit. The presence of adverse events was evaluated using a self-report diary also and by clinical interview and exploring every visit throughout the study, and they were reported to the ethics committee.

Randomization

At recruitment, participants were randomized to one of the two intervention groups by random numbers in sealed envelopes generated by a table of random numbers.

Blinding

Patients and researchers or staff involved in outcome assessment were blinded to treatment. Blinding of patients and researchers was maintained until completion of the analyses.

Sample size

Sample size was determined based on the formula for clinical trials.\(^16\) A total of 12 patients per group are estimated to be necessary to achieve a confidence level of 95% and a statistical power of 80%, taking into account a standard deviation and expected difference with regard to previous
results of metabolic syndrome and insulin sensitivity, and considering 20% more for possible losses.

Statistical analysis

Values are expressed in accordance with the International System of Units and are presented as mean and standard deviation. Nonparametric statistics were used. Differences in the means of continuous variables in the group of patients treated with ursolic acid compared with the group of patients treated with placebo were carried out using the Mann–Whitney U Test for independent samples. Differences in the means of continuous variables in the group of patients treated with ursolic acid compared with the group of patients treated with placebo were carried out using the Wilcoxon signed-rank test for dependent samples. The differences in the means of continuous variables measured at the beginning and at the end of the study were carried out using the Wilcoxon signed-rank test for dependent samples. The differences in nominal variables were analyzed using the Chi-square ($\chi^2$) test. A $P$-value ≤ .05 was considered statistically significant. The analysis was performed using the statistical package SPSS version 21 for Windows.

Ethical considerations

The study was evaluated and approved by an institutional ethics committee (CEI/075/2014), and written informed consent was obtained from all volunteers.

Clinical trial registration

NCT02337933

RESULTS

All patients completed 12 weeks of pharmacological intervention with a treatment adherence >90%. The mean age of patients was 46.5 ± 8.7 and 43.7 ± 8.5 years in the placebo and ursolic acid group, respectively, without a significant difference between groups ($P = .478$).

There were no significant differences between groups at baseline in all measurements.

After the intervention, transient remission of metabolic syndrome was achieved in 50% of patients treated with ursolic acid, whereas in the control group, no patients achieved remission ($P = .005$).

Characteristics before and after the pharmacological intervention are shown in Table 1. Ursolic acid achieved a significant decrease in body weight (75.7 ± 11.5 vs. 71 ± 11 kg, $P = .002$) and BMI (29.9 ± 3.6 vs. 24.9 ± 1.2 kg/m², $P = .049$), as well as waist circumference (93 ± 8.9 vs. 83 ± 8.6 cm, $P = .008$) and fasting plasma glucose (6.0 ± 0.5 vs. 4.7 ± 0.4 mmol/L, $P = .002$) when comparing baseline versus 12 weeks of the intervention. However, there were no differences in lipid profile and blood pressure. There was a significant increase in insulin sensitivity (3.1 ± 1.1 vs. 4.2 ± 1.2, $P = .003$) after administration of ursolic acid. The parameters of inflammation, including IL-6 and CRP, were not changed.

There were no significant differences in any of the measurements after placebo administration.

Ursolic acid was well tolerated. The presence of adverse events was similar between the two groups of intervention and there was no statistically significant difference between them. Abdominal distention (16.6 vs. 25.0%, $P = .615$), diarrhea (8.3 vs. 16.6%, $P = .537$), and flatulence (16.6 vs. 8.3%, $P = .537$) were observed in patients treated with placebo and ursolic acid, respectively. All adverse events were classified as mild, with a duration of less than a day. There were no serious adverse events.

Table 1. Characteristics Before and After Pharmacological Intervention

<table>
<thead>
<tr>
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<th>Placebo (n = 12)</th>
<th>Ursolic acid (n = 12)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>81.5 ± 14</td>
<td>81 ± 15</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.7 ± 4.2</td>
<td>32.1 ± 3.9</td>
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<tr>
<td>Metabolic syndrome components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference  $\delta$ (cm)</td>
<td>97 ± 10</td>
<td>96 ± 8</td>
</tr>
<tr>
<td>Waist circumference  $\gamma$ (cm)</td>
<td>111 ± 1</td>
<td>108 ± 6</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>128 ± 20</td>
<td>124 ± 15</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80 ± 10</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>Fasting glucose (0') (mmol/L)</td>
<td>5.8 ± 0.9</td>
<td>5.7 ± 0.7</td>
</tr>
<tr>
<td>Postload glucose (120') (mmol/L)</td>
<td>8.3 ± 1.8</td>
<td>8.5 ± 1.9</td>
</tr>
<tr>
<td>Tryglicerides (mmol/L)</td>
<td>2.5 ± 0.1</td>
<td>2.5 ± 1.3</td>
</tr>
<tr>
<td>HDL-c $\delta$ (mmol/L)</td>
<td>1.8 ± 0.7</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>HDL-c $\gamma$ (mmol/L)</td>
<td>0.9 ± 0.3</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matsuda index</td>
<td>3.1 ± 0.6</td>
<td>2.6 ± 1.4</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
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<tr>
<td>IL-6 (pg/mL)</td>
<td>0.34 ± 0.30</td>
<td>0.31 ± 0.20</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.04 ± 0.03</td>
<td>0.03 ± 0.03</td>
</tr>
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</table>

Significant differences baseline versus 12 weeks by group: ** $P < .005$, * $P < .05$ (P-value obtained by Wilcoxon rank test).

BMI, body mass index; CRP, C reactive protein; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein cholesterol; IL-6, interleukin-6; SBP, systolic blood pressure.
DISCUSSION

Despite the great effort that has been made to treat the metabolic syndrome, the effectiveness of many treatments is controversial and its prevalence continues to increase.\(^1\)\(^2\) Given the increased risk of CVD and T2DM in patients with metabolic syndrome, it is necessary to find an appropriate treatment that will prevent the onset of both diseases through the modification of one or more of its components. Even though the administration of ursolic acid has shown positive in vitro and in vivo effects on lipid and glucose metabolism,\(^7\)-\(^14\) this study represents the first clinical trial that evaluates the effect of ursolic acid on all the components of the metabolic syndrome as a whole.

In our study, ursolic acid achieved transient remission of metabolic syndrome in half of the patients treated, whereas in the placebo group, no patient was able to achieve remission. As was already mentioned, remission of metabolic syndrome is considered when individuals no longer meet the diagnostic criteria because of the improvement in some component of the syndrome. This is important because each component of the metabolic syndrome is a cardiovascular risk factor, so we can ensure that the cardiovascular risk is lower in patients treated with ursolic acid, by the end of the study. Components of metabolic syndrome that improved further in our study were waist circumference and fasting glucose, and there was also a significant decrease of body weight and BMI.

Body weight was decreased significantly with the administration of ursolic acid, so BMI was reduced as well. A change of \(\sim 5\) kg was observed in the ursolic acid group, whereas in the placebo group, the decrease was almost nil. This finding has a considerable clinical relevance, because weight loss in patients treated with ursolic acid represented an average decrease of 8% with respect to the basal body weight, which falls within the objectives of the first-line treatment for metabolic syndrome by the IDF, which promotes body weight loss of 5 to 10%.\(^2\) In addition, BMI also decreased in the ursolic acid group. In some cases, this reduction represented a change in the classification of obesity according to the BMI of obesity grade I to overweight, which is a significant improvement at the metabolic level. Our results are consistent with other studies that also noted a decrease in body weight,\(^17\)\(^19\)\(^20\) or it was considered as a protective factor against weight gain with a high-fat diet.\(^19\)

These findings can be explained by the existing evidence in the literature regarding a reduction of adipogenesis.\(^13\) Furthermore, interestingly in other studies with ursolic acid, an increase in lipolysis by activating protein kinase A\(^14\) was observed, which can augment also muscle and brown adipose tissue, promoting thermogenesis and energy expenditure. Although there was no increase in muscle mass in our study, we did not evaluate brown adipose tissue, which represents an interesting measurement to perform in futures studies, as well as measurements of energy expenditure.

Our study shows a significant reduction of waist circumference, which is clinically important in metabolic syndrome because it represents the basis of the diagnosis, and the improvement in this component represents a major advance in the treatment of this syndrome. In addition, waist circumference is considered an indirect measure of abdominal obesity and, therefore, of visceral fat, and there is evidence for this type of obesity resulting in increased cardiovascular risk, which is precisely what we are trying to prevent when we talk about the treatment of the metabolic syndrome. In this sense, a study on rats showed a decrease in visceral fat after administration of ursolic acid.\(^19\) It would be interesting in an upcoming study to measure, in addition to the waist, visceral fat or even subcutaneous fat.

A decrease in fasting glucose concentrations after administration of ursolic acid was observed, unchanged on postload glucose concentrations. The magnitude of the change was \(1.3 \pm 0.5\) mmol/L on fasting glucose in the ursolic acid group, and this was significantly higher than that in the placebo group. This significant finding in glycemia has a considerable clinical importance because this disturbance known as impaired fasting glucose is considered one of the first alterations in glucose homeostasis foregoing T2DM, called prediabetes that if treated can reverse the progression of the disease, hence the importance of treating this precondition of T2DM. Although there are possible explanations for improvement of glycemia by ursolic acid, such as the inhibition of \(\alpha\)-amylase enzyme,\(^9\) the decrease in hydrolysis and starch absorption, or a decrease in the polyol pathway or inhibition of aldose reductase, thereby decreasing the glucotoxicity,\(^11\) it is likely that the best explanation in this case, is that it is a consequence of increased insulin sensitivity through actions of ursolic acid on the insulin receptor,\(^7\) as discussed in detail hereunder, and this promotes GLUT4 translocation.\(^8\)

We did not observe a difference in lipid profile, including triglycerides, total cholesterol, and HDL-c circulating concentrations. This despite the fact that there are numerous studies that describe actions of ursolic acid such as the decrease of pancreatic lipase,\(^12\) which would reduce the absorption of lipids, as well as an increase in lipolysis,\(^13\) producing an increase in their use. Probably with a longer intervention period, there could be a significant difference in serum lipids.

Ursolic acid did not affect both DBP and SBP in our study. The improvement of blood pressure by ursolic acid has been reported in studies in animal models of hypertension sensitive to salt. This improvement induced by ursolic acid was related to inhibition of the reabsorption of sodium and potassium in the early portion of the distal tubule in rats.\(^20\) There are publications that promote the use of ursolic acid as an adjunctive treatment of hypertension, relating this indirectly with effects of ursolic acid as hypoglycemic, lipid-lowering agent, and as an antioxidant.\(^17\)\(^20\) In our study, as we have mentioned, a hypoglycemic effect was observed, but we saw no lipid-lowering effect, and we consider that it would be interesting to assess its antioxidant effects, because the components of this syndrome are related to oxidative stress.

In our study, ursolic acid was able to increase insulin sensitivity. Although the gold standard for the measurement
of insulin sensitivity is the euglycemic–hyperinsulinemic clamp, Matsuda index that was used in this study has a good correlation of 0.73 (P < .0001). Our favorable outcomes on insulin sensitivity are consistent with a large number of studies that attempt to explain the activity of ursolic acid as hypoglycemic,10–12 and other in vitro studies that demonstrate the action of ursolic acid that increases the number of insulin receptors and the number of activated receptors by inhibiting protein tyrosine phosphatase 1B, an enzyme related to downregulation of the insulin receptor.

In our attempt to assess the effect of ursolic acid on inflammation, we found no significant differences in concentrations of IL-6 and PCR before and after the intervention. Even though ursolic acid properties have shown an anti-inflammatory effect,6 in our study we could not see this effect, probably because studies showing this effect were performed in greater inflammatory conditions such as cancer and liver diseases, including hepatitis or cirrhosis. In our case, the patients were overweight or obese, which is an inflammatory disease, but this inflammatory condition is lower and chronic, which may explain that such marked anti-inflammatory effect was not observed.

These findings may be the basis for proposing the administration of ursolic acid as a treatment option for patients with metabolic syndrome. Long-term studies with larger sample sizes will be necessary to confirm our results.

In conclusion, our data show that the administration of ursolic acid for 12 weeks achieved transient remission of the metabolic syndrome in half of the patients. It significantly reduced body weight, BMI, waist circumference, and fasting glucose concentrations and it significantly increased insulin sensitivity.

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No competing financial interests exist.

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