Isohumulones, Bitter Acids Derived from Hops, Activate Both Peroxisome Proliferator-activated Receptor α and γ and Reduce Insulin Resistance*

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The peroxisome proliferator-activated receptors (PPARs) are dietary lipid sensors that regulate fatty acid and carbohydrate metabolism. The hypolipidemic effects of fibrate drugs and the therapeutic benefits of the thiazolidinedione drugs are due to their activation of PPARα and -γ, respectively. In this study, isohumulones, the bitter compounds derived from hops that are present in beer, were found to activate PPARα and -γ in transient co-transfection studies. Among the three major isohumulone homologs, isohumulone and isocohumulone were found to activate PPARα and -γ. Diabetic KK-A'/ mice that were treated with isohumulones (isohumulone and isocohumulone) showed reduced plasma glucose, triglyceride, and free fatty acid levels (65.3, 62.6, and 73.1%, respectively, for isohumulone); similar reductions were found following treatment with the thiazolidinedione drug, pioglitazone. Isohumulone treatment did not result in significant body weight gain, although pioglitazone treatment did increase body weight (10.6% increase versus control group). C57BL/6N mice fed a high fat diet that were treated with isohumulones showed improved glucose tolerance and reduced insulin resistance. Furthermore, these animals showed increased liver fatty acid oxidation and a decrease in size and an increase in apoptosis of their hypertrophic adipocytes. A double-blind, placebo-controlled pilot study for studying the effect of isohumulones on diabetes suggested that isohumulones significantly decreased blood glucose and hemoglobin A1c levels after 8 weeks (by 10.1 and 6.4%, respectively, versus week 0). These results suggest that isohumulones can improve insulin sensitivity in high fat diet-fed mice with insulin resistance and in patients with type 2 diabetes.

Type 2 diabetes represents a heterogeneous group of disorders characterized by increased insulin resistance. A sedentary lifestyle, fatty diet, obesity, and increased age have all been associated with the development of insulin resistance, although its molecular basis is still unknown. Thiazolidinediones were shown to improve insulin sensitivity in various animal models of diabetes (1) and are commonly used to treat type 2 diabetes. Thiazolidinediones directly bind to, and activate, the transcriptional factor peroxisome proliferator-activated receptor (PPAR)γ. PPARγ is highly expressed in adipocytes where it regulates the genes responsible for growth and differentiation following activation by both natural and synthetic ligands. Activation of PPARγ was shown to not only stimulate the differentiation of adipocytes but to also induce their apoptotic death, thereby preventing adipocyte hypertrophy (2). PPARα, another nuclear fatty acid receptor that is widely expressed in the liver, muscle, kidney, and intestine mediates the expression of genes involved in lipid metabolism. Activators of PPARs, such as fibrates, lower circulating lipid levels and are commonly used to treat hypertriglyceridemia and other dyslipidemic states. Abnormalities in fatty acid metabolism underlie the development of insulin resistance and alterations in glucose metabolism. Recent studies (3, 4) suggested that the activation of PPARα improved the insulin resistance that was triggered by the overeffect and accumulation of lipid. It has also been reported (5, 6) that several novel compounds that act as co-ligands for PPARα and -γ can improve insulin sensitivity and correct diabetic dyslipidemia in obese diabetic animals. Attention has recently focused on the potential use of constituents in plants and other foods for the treatment of diabetic symptoms (7). The treatment of type 2 diabetes with herbal plants, which was carried out generations ago in Europe, may have provided some benefit because they contained compounds that stimulated the activity of PPARα and -γ (8, 9). Hops, the female inflorescences of the hop plant (Humulus lupulus L.), are used as a preservative and flavoring in beer. Isohumulones, called iso-α acids, are the compounds that impart the bitter flavor to beer. Specifically, they are converted from humulones, i.e. α acids, derived from hops by isomerization during the brewing process. Humulone was shown to inhibit angiogenesis by suppressing cyclooxygenase-2, one of the key enzymes involved in carcinogenesis (10, 11); other than having antibacterial properties, no physiological effects were reported for iso-umulones (12).

In this study, we showed that isohumulones can activate PPARα and -γ in vitro co-transfection assays. We further...
demonstrated that the treatment of mildly diabetic mice and patients with isohumulones improved their insulin sensitivity.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Isomerized hop extract (IHE, ISOHOPCON2) was purchased from Echigo Hop Products Co., Ltd. (Kent, UK). IHE containing isohumulones at a purity of 79%. Isohumulone, isocohumulone, and isoadohumulone were purified from IHE by centrifugal partition chromatography. Specifically, IHE was neutralized to pH 7.0 with acetic acid and subjected to centrifugal partition chromatography (25°C, 600 rpm). Ethyl acetate and 0.1 M ammonium acetate were used as the stationary and mobile phases, respectively. Fractions isolated were adjusted to pH 3.0 with HCl, after which they were extracted twice with ethyl acetate. The extracts were washed twice with saturated NaCl and the purified isohumulones dried to determine their total yields, after which they were dissolved in ethanol. Pogliatzone was purified from Actos (Takeda Chemical Industries Ltd., Osaka, Japan) by extraction with chloroform and methanol. Fenofibrate was purchased from Sigma.

**Transient Transfection Assay**—An expression plasmid containing the ligand binding domain of human PPARγ fused to the GAL4 DNA binding domain was constructed as described previously (13, 14). The plasmid encoding for the full-length human PPARγ and its variants that can complicate the interpretation of the results, were included in this study. Ten men and ten women were randomized into one of two groups receiving either placebo or a capsule containing 100 mg/kg weight, or pioglitazone at a dose of 10 mg/kg weight, for 10 weeks as described previously (17). Some of these animals were included in this study. Ten men and ten women were randomized into one of two groups receiving either placebo or a capsule containing 100 mg/kg weight, or pioglitazone at a dose of 10 mg/kg weight, for 10 weeks after which their subcutaneous white adipose tissue was removed and fixed in 4% paraformaldehyde in phosphate-buffered saline. Fixed specimens were dehydrated, embedded in tissue-freezing medium (Tissue Tek OCT compound; Sakura Finetechnical Co., Ltd., Tokyo, Japan), and involved in lipid uptake and storage, were also used for the analysis. The following sense and antisense primers were used (GenBank accession numbers are in parentheses): 36B4 (X15267), nucleotides 740–759 and 907–929; adipo-236 and 364–365; LIPA (AF006888), nucleotides 1392–1415 and 1607–1628; FAT (L23108), nucleotides 331–352 and 509–531.

**Acyl-CoA Oxidase Activity**—Activity of ACO was determined as described previously (22). Six-week-old female C57BL/6N mice were maintained on a high fat diet for 10 weeks as described previously (17). Some of these animals were included in this study. Ten men and ten women were randomized into one of two groups receiving either placebo or a capsule containing 100 mg/kg weight, or pioglitazone at a dose of 10 mg/kg weight, for 10 weeks after which their subcutaneous white adipose tissue was removed and fixed in 4% paraformaldehyde in phosphate-buffered saline. Fixed specimens were dehydrated, embedded in tissue-freezing medium (Tissue Tek OCT compound; Sakura Finetechnical Co., Ltd., Tokyo, Japan), and involved in lipid uptake and storage, were also used for the analysis. The following sense and antisense primers were used (GenBank accession numbers are in parentheses): 36B4 (X15267), nucleotides 740–759 and 907–929; adipo-236 and 364–365; LIPA (AF006888), nucleotides 1392–1415 and 1607–1628; FAT (L23108), nucleotides 331–352 and 509–531.

**Statistical Analysis**—Results are expressed as the means ± S.D. The data in the animal experiments were analyzed by nonrepeated measures analysis of variance followed by a Dunnett’s test. Significance was assumed if the p value was <0.05.
Isohumulones Improve Insulin Resistance

Fig. 1. Structure of isohumulone. Isohumulone (R: –CH\(_2\)CH–(CH\(_3\))\(_2\); 2-(3-methylbutanoyl)-5-(3-methyl-2-butenyl)-3,4-dihydroxy-4-(4-methyl-3-pentenyl)-2-cyclopentenone), isoohumulone (R: –CH(CH\(_3\))\(_2\); 2-(2-methylpropanoyl)-5-(3-methyl-2-butenyl)-3,4-dihydroxy-4-(4-methyl-3-pentenyl)-2-cyclopentenone), and isoadhumulone (R: –CH(CH\(_3\))C\(_2\)H\(_5\); 2-(2-methylbutanoyl)-5-(3-methyl-2-butenyl)-3,4-dihydroxy-4-(4-methyl-3-pentenyl)-2-cyclopentenone).

Fig. 2. Isohumulone-induced PPAR transactivation. A, CV-1 cells were co-transfected with a luciferase reporter plasmid, pG5 luc, containing five copies of GAL4 upstream activating sequence in the promoter region and an expression vector for the human PPAR\(_\gamma\) ligand binding domain fused to the GAL4 DNA binding domain. Results are the relative luciferase expression levels normalized with the protein concentration of the cell lysates. B, HepG2 cells were co-transfected with pG5 luciferase, and an expression vector for the human PPAR\(_{\alpha}\) coding region fused to the GAL4 DNA binding domain. Isohumulone (IH), isoohumulone (I\(\alpha\)H), and isoadhumulone (I\(\alpha\)H) at the indicated concentrations were added to the transfected cells. Pioglitazone (Pio) and fenofibrate (Feno) were used as positive controls for PPAR\(_\gamma\) and PPAR\(_{\alpha}\) transactivation, respectively.

with a reporter plasmid containing the reporter luciferase gene with five GAL4 binding sites in the promoter region. In the case for PPAR\(_{\alpha}\), a chimeric plasmid encoding the full-length of PPAR\(_{\alpha}\) protein fused to the DNA binding domain of GAL4 was used, because the chimeric construct containing PPAR\(_{\alpha}\) ligand binding and GAL4 DNA binding domains did not provide enough luciferase activity in the presence of a PPAR\(_{\alpha}\) agonist, fenofibrate (data not shown). Furthermore, human hepatoma cells, HepG2, instead of CV-1 were used for the assay to obtain a higher sensitivity.

A dose-dependent increase in luciferase activity was observed following the addition of isohumulones to cells transfected with the PPAR\(_\gamma\)-GAL4 chimera construct. The addition of either 10 \(\mu\)M isohumulone, isoohumulone, or isoadhumulone induced a 3.8-, 3.5-, and 2.8-fold increase in luciferase activity, respectively, compared with the vehicle control (Fig. 2A). These activities were almost the same as those seen when 1 \(\mu\)M pioglitazone, a specific agonist of PPAR\(_\gamma\), was added to the cells. Isohumulone and isoohumulone also activated the PPAR\(_{\alpha}\)/GAL4 chimera construct in a dose-dependent manner; 10 \(\mu\)M isohumulone and isoohumulone increased luciferase activity by about 3.2- and 1.9-fold, respectively, compared with the vehicle control (Fig. 2B). Isoadhumulone had no effect on the PPAR\(_{\alpha}\)/GAL4 chimera construct, suggesting that the side chain of isohumulone may be involved in the activation of the PPAR\(_{\alpha}\) receptor. The activity of isohumulone at 30 \(\mu\)M was almost the same as that of 3 \(\mu\)M fenofibrate, a PPAR\(_{\alpha}\)-selective agonist.

Isohumulones Prevent the Development of Diabetes in KK-A\(^{-}\)Mice—Treatment of KK-A\(^{-}\) mice with isohumulones for 2 weeks significantly lowered their plasma triglyceride (62.6% for isohumulone and 76.4% for isoohumulone, respectively) and free fatty acid levels (73.1% for isohumulone and 84.8% for isoohumulone, respectively). These reductions were comparable with those seen in 0.05% pioglitazone-treated mice (60.5 and 69.9%, respectively) (Fig. 3A and B). Nonfasting plasma glucose levels of isohumulones-treated mice were also reduced to 65.3% of controls for isohumulone and 87.1% of controls for isoohumulone, although they were higher than those seen in animals fed pioglitazone (35.1%) (Fig. 3C). Most interesting, there was a significant difference in body weight gain between these groups despite their equivalent caloric intake. Thus, pioglitazone induced a 10% increase in body weight, whereas isohumulones did not induce an increase in body weight compared with the control group (Fig. 3D).
Isohumulones Reduced Insulin Resistance in High Fat Diet-fed C57BL/6N Mice—Female C57BL/6N mice were fed a high fat diet for 12 weeks. On their last 14 days on the diet, they were orally administered isoohumulone. OGTTs and ITTs were performed on their 10th and 14th day of isoohumulone treatment, respectively. After glucose loading, plasma glucose levels in the mice treated with isoohumulone at 10 and 100 mg/kg/day were significantly reduced compared with mice treated with vehicle at all time points except for the mouse treated with 100 mg/kg of isoohumulone at 120 min (Fig. 4A). Fasting plasma insulin levels were significantly reduced in animals treated with 100 mg/kg isoohumulone (2396 ± 520 and 1605 ± 570 (pg/ml) for vehicle-treated and 100 mg isoohumulone-treated mice, respectively). Plasma insulin levels during the OGTT were also reduced in the isoohumulone-treated animals (Fig. 4B). The insulin resistance indices of mice treated with isoohumulone (1094.4 ± 259.2 and 1034.0 ± 259.2 for 10 mg of isoohumulone-treated and 100 mg of isoohumulone-treated mice, respectively) were significantly (p < 0.01 using the Dunnett’s test) lower than that seen in the control group (2217.3 ± 792.9), indicating that isoohumulone improved insulin sensitivity in mice fed a high fat diet. Improvement in insulin sensitivity was also observed by the treatment with isoohumulone; significant decrease in IR was observed in the group receiving 100 mg/kg of isoohumulone (1240.2 ± 259.5, p < 0.01 using the Dunnett’s test, data not shown). Results of the ITT showed a greater glucose lowering effect in mice treated with isoohumulone for 10 days than in vehicle-treated animals (Fig. 4C). Plasma glucose levels in mice treated with 5 or 50 mg/kg of isoohumulone were significantly reduced to 76.8 and 69.3% of controls after 60 min and to 82.8 and 69.3% of controls after 120 min.

Isohumulones Up-regulated the Expression of Genes Involved in Fatty Acid Oxidation in the Liver—Quantitative real time RT-PCR analysis of the mRNAs for ACO and fatty acid translocase/CD36 (FAT) genes in the liver of KK-A’ mice treated with isoohumulone, isoohumulone, or pioglitazone were performed (Fig. 5A). ACO and FAT, whose expressions are regulated by PPARα, are involved in peroxisomal β-oxidation of fatty acids and in the uptake of long chain fatty acids through the cell membrane, respectively (24, 25). Treatment of mice with isoohumulone, isoohumulone, or pioglitazone increased ACO and its mRNA levels by 1.6-, 1.7-, and 2.7-fold, and FAT mRNA levels by 2.9-, 3.3-, and 2.8-fold, compared with the control mice, respectively. The significant increase in ACO and FAT mRNA levels in the pioglitazone-treated mice was likely due to activation of PPARα by pioglitazone because induction of PPARα/GAL4 chimera transactivation with pioglitazone was observed in our reporter assay (data not shown). An increase in the activity of ACO was observed in the liver of C57BL/6N mice treated with isoohumulone or isoohumulone (Fig. 5B).

Isohumulones Reduced Adipocyte Hypertrophy in White Adipose Tissue—Isohumulone treatment unexpectedly resulted in only a fairly small increase in the expression of PPARγ-regulated adipocyte-ADRP and LPL genes, which are involved in lipid uptake and storage in the epididymal white adipose tissue (WAT) (26) of KK-A’; only the LPL mRNA level in the mice treated with isoohumulone significantly increased by 1.2-fold, although treatment with pioglitazone did increase the adipocyte-ADRP and LPL mRNA levels by 2- and 1.7-fold, respectively (Fig. 5C). Histological analysis of subcutaneous WAT of the high fat diet-fed C57BL/6N mice treated with isoohumulone, isoohumulone, or pioglitazone for 10 days revealed an increase in the number of small adipocytes by treatment with all three of these compounds compared with vehicle-treated mice (Fig. 5D). We also examined whether isoohumulones induced apoptosis in adipocytes. Our results indicated that the treatment of high fat diet-fed mice with isoohumulones for 10 days induced the apoptosis of hypertrophic adipocytes, which was also observed in the mice treated with pioglitazone (Fig. 5E).

The Effects of Oral Isohumulones in Type 2 Diabetics in a Placebo-controlled Pilot Study—The ability of IHE to transactivate PPARα and -γ and to improve glucose tolerance in KK-A’ mice was identical to that seen with isoohumulones isolated from the extract (data not shown). Supplementation of 10 mild diabetic patients with IHE over 8 weeks resulted in a significant reduction in their blood glucose and hemoglobin A1c levels by 10.1 and 6.4%, respectively; most interesting, a weak but significant reduction in blood glucose levels (7.3%) was also observed in the placebo control group. Furthermore, significant reductions in systolic blood pressure and blood levels of glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, and γGTP were observed in the IHE-treated group (7.2, 33.4, 21.3, and 25.6% reduction, respectively) (Table I).

DISCUSSION

Metabolic syndrome, which is particularly relevant to insulin resistance, is characterized by glucose intolerance, hyperinsulinemia, dyslipidemia, and hypertension and is frequently associated with visceral obesity. The clustering of these multiple cardiovascular risk factors increases the risk of developing atherosclerotic vascular disease. Pharmacological treatment of this syndrome aims to reduce insulin resistance and other risk factors.
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Fig. 5. Effects of isohumulone and isocohumulone on liver and white adipose tissue. A and C, total RNA was isolated from liver and subcutaneous adipose tissue of KK- 
A mice fed diets containing 0.18% isohumulone (IH), 0.18% isocohumulone (IcH), or 0.05% pioglitazone (pio). Quantitative RT-PCR was performed to measure the mRNA levels of liver ACO and FAT (A), and adipocyte-ADRP and LPL in white adipose tissue (WAT) (C). LPL, lipoprotein lipase. Results are presented as relative expression levels normalized to the expression in the control (Cont) group (mean ± S.D., n = 6). B, ACO activity in the liver of C57BL/6N mice treated with isohumulone or isocohumulone at 100 mg/kg weight for 6 days. Data are presented as the amount of hydrogen peroxide produced by oxidation of palmitoyl-CoA in the homogenates. *, p < 0.05; **, p < 0.01 versus control (Ctrl). D, histological analysis and cell-size distribution of subcutaneous WAT in C57BL/6N mice. High fat diet-fed mice were orally administered vehicle (Cont), isohumulone, isocohumulone (100 mg/kg weight), or pioglitazone (Pio) (10 mg/kg weight) for 2 weeks. E, terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling staining of representative sections of white adipose tissue from D. Bar indicates 100 μm.

Table I

Results of the placebo-controlled pilot study of the effects of hop extract in type 2 diabetic patients

<table>
<thead>
<tr>
<th>Hop extract</th>
<th>Placebo</th>
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<tr>
<td></td>
<td>0 week</td>
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<tr>
<td>Blood glucose (mg/dl)</td>
<td>127.1 ± 10.9</td>
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<tr>
<td>HbA1c (%)</td>
<td>7.14 ± 0.36</td>
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<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>137.1 ± 13.6</td>
</tr>
<tr>
<td>GPT (IU/liter)</td>
<td>40.8 ± 26</td>
</tr>
<tr>
<td>GOT (IU/liter)</td>
<td>28.6 ± 13</td>
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<tr>
<td>γGTP (IU/liter)</td>
<td>48.1 ± 41</td>
</tr>
</tbody>
</table>

* p < 0.01 versus 0 week of each group.

† p < 0.05.

§ GPT indicates glutamic pyruvic transaminase.

\* GOT indicates glutamic oxaloacetate transaminase.

Factors by modulating PPARs, nuclear hormone receptors that play a role in regulating energy metabolism. Fibrate drugs, which act as ligands for PPARα and thiazolidinedione drugs, which are ligands for PPARγ, are often used to treat hyperlipidemia and hyperglycemia, respectively. The combination therapy of these medications is an attractive option for the treatment of obese type 2 diabetics (10, 11). Compounds that have dual agonistic activity on both of these receptors have been shown to improve insulin sensitivity and dyslipidemia in obese diabetic animals (6, 27). In the present study, we showed that isohumulones, the bitter compounds present in beer, activated PPARα and γ and mediated insulin resistance and dyslipidemia in diabetic animals and reduced hyperglycemia in patients with type 2 diabetes.

Our experiments using an in vitro reporter assay indicated that isohumulones activated transcription that was mediated by PPARα and PPARγ, suggesting that isohumulones may modulate reactions that are regulated by these transcriptional activators. When KK-A' mice, a model of noninsulin-dependent diabetes, were treated with isohumulone and isocohumulone, their hyperglycemia and hyperlipidemia improved, as they also did following treatment with pioglitazone, a thiazolidinedione drug. However, treatment did not cause significant body weight gain nor marked increases in mRNA levels of PPARγ-regulated adipocyte-ADRP and LPL genes, which are involved in lipid uptake and storage in adipose tissue, in contrast to pioglitazone. It has been suggested that Fmoc-L-leucine, a potent insulin-sensitizing compound, acts as a selective PPARγ modulator and improves insulin resistance in diabetic mice despite only marginally inducing adipocyte-ADRP and LPL gene expression in adipose tissue (28). It should be noted that treatment with this compound also had no effect on body...
weight. A low affinity PPARγ antagonist, i.e. LG100,641, has also been shown to improve insulin sensitivity in 3T3-L1 adipocytes in vitro without markedly up-regulating PPARγ-mediated gene expression and adipocyte differentiation (29). Thus, it is likely that an increase in body weight and up-regulation of PPARγ-mediated gene expression in adipose tissue are not always observed in rodents treated with PPARγ agonists.

Mice fed a high fat diet develop hyperglycemia and obesity and are used as a model of noninsulin-dependent diabetes mellitus (17). Insulin resistance and glucose intolerance in high fat diet-fed obese mice improved after short term oral administration of isohumulone and isocoumarone. Their adipocyte hypertrophy also responded to treatment, with an increase seen in the number of apoptotic adipocytes in the isohumulone-treated groups; similar results were found with pioglitazone. It has been suggested that treatment of obese diabetic mice with thiazolidinediones might stimulate adipocyte differentiation and apoptosis and thereby prevent adipocyte hypertrophy. In fact, such treatment was shown to be associated with reduced insulin resistance that may have been due to reductions in free fatty acid and tumor necrosis factor-α levels, and up-regulation of adiponectin (2, 30). Taken together, these results suggest that isohumulones improve insulin sensitivity by acting on adipose tissues.

In our in vitro reporter assay results showed that isohumulone and isocoumarone activated PPARα-mediated gene expression. It was therefore likely that the increase in the mRNA levels of ACO and fatty acid translocase/CD36 genes, which are involved in fatty acid oxidation, in the liver of KK-A1 mice treated with isohumulones was due to the activation of PPARα. The mRNA for medium chain acyl-CoA dehydrogenase, an enzyme involved in fatty acid β-oxidation, was also increased in the liver by this treatment (data not shown). An increase in the enzymatic activity of ACO was observed in the liver of C57BL/6N mice treated with isohumulones. These results may indicate that isohumulones facilitate lipid metabolism in the liver. It has been reported that fenofibrate reduced plasma insulin and glucose levels in high fat diet-induced C57BL/6 mice and obese Zucker and OLETF rats, whereas it prevented weight gain and expansion of adipose tissue mass (4, 31). The activation of hepatic β-oxidation in isohumulone-treated obese C57BL/6N mice may have been the mechanism by which adipocyte hypertrophy was reduced and at least partly responsible for the amelioration of insulin resistance. However, it should be noted that no improvement in hyperglycemia and hyperlipidemia was observed in KK-A1 mice that were treated with fenofibrate, suggesting that activation of PPARα alone was not sufficient to ameliorate insulin resistance in this model (data not shown). Anti-diabetic effects of dual PPARα and PPARγ agonists have been reported over the past several years. Raggagazir, a PPARα and -γ agonist, was shown to reduce plasma triglyceride and glucose levels in high fat diet-fed animals in the absence of weight gain (5); similar results were observed in Zucker fa/fa rats (32). Another PPARα/γ co-agonist, KRP-297, was shown to reduce plasma glucose and insulin levels in ob/ob and db/db mice (22). The co-activation of PPARα and -γ by isohumulones, leading to the activation of fatty acid β-oxidation in the liver and the normalization of adipocyte hypertrophy, may be responsible for the amelioration of insulin resistance in diabetic mice. Recently, it was reported (33, 34) that the activation of PPARα increased fatty acid oxidation and resulted in the prevention of obesity. It should be noted that the activation of PPARα with isohumulones was not observed in in vitro reporter assays (data not shown).

Natural constituents in plants that are present in folk remedies as well as traditional foods have been reported to be effective for the treatment and prevention of lifestyle-related diseases (7, 35). A recent study demonstrated that the plant sterol guggulsterone, derived from Commiphora mukul (guggul in Sanskrit), which has been used in Ayurvedic medicine, acts as an antagonist for the nuclear hormone receptor, farnesoid X receptor, and lowers low density lipoprotein levels in rodents and humans (36). Hops, which have been used as an herbal medicine for many years, were shown to contain several compounds that have significant biological effects, such as 8-precyanidin, which has estrogenic activity (37), and xanthohumol and hexahydrocolupulone which were shown not to inhibit the growth of cancers (38). Humulone was also shown to have anti-tumor effects (10, 11, 39). Isohumulones, which are isomerized humulones, are present in beer. To date, most studies of the therapeutic effects of isohumulones have focused on their ability to inhibit bacterial growth (12). In our study, isohumulone and isocoumarone were shown to activate PPARα and -γ, whereas isoadhumulone only activated PPARγ; the reason for the differing effects of these compounds is unclear. Further studies are required to determine the molecular basis of the interaction between isohumulones and PPARα.

In conclusion, our results suggest that isohumulones, the bitter compounds derived from hops in beer, activate both PPARα and -γ and improve insulin sensitivity and lipid metabolism. The simultaneous activation of PPARα and -γ with isohumulones may be an effective approach for the treatment of metabolic syndrome in which glucose and lipid metabolism are both impaired. Results of a preliminary clinical study indicated that isohumulones improved insulin sensitivity in type 2 diabetic patients. Most interesting, in this latter study, patients also showed a significant reduction in systolic blood pressure and in the levels of serum markers of liver disorder. Thus, isohumulones in beer are worth investigating further as therapeutic agents for the treatment of metabolic syndrome associated with insulin resistance.

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REFERENCES


