International Journal of Obesity (2005) 29, 991–997
 0307-0565/05 $30.00
www.nature.com/ijo

PAPER

Prevention of diet-induced obesity by dietary isomerized hop extract containing isohumulones, in rodents

H Yajima[1]*, T Noguchi[1], E Ikeshima[1], M Shiraki[1], T Kanaya[1], N Tsuboyama-Kasaoka[2], O Ezaki[2], S Oikawa[3] and K Kondo[1]

[1]*Central Laboratories for Key Technology, Kirin Brewery Co., Ltd, Kanagawa, Japan;* [2]*Division of Clinical Nutrition, National Institute of Health and Nutrition, Tokyo, Japan; and* [3]*Department of Medicine, Nippon Medical School, Tokyo, Japan*

OBJECTIVE: Isomerized hop extract (IHE), which consists mainly of isohumulones and is required in the beer brewing process, was investigated for its effects on diet-induced obesity in two strains of mice.
DESIGN: C57BL/6N and KK-A^y mice were fed a standard or high-fat diet containing IHE and their body and tissue weights were measured at various time points. Oral glucose tolerance tests (OGTT) and insulin tolerance tests (ITT) were carried out in high-fat diet-fed C57BL/6N mice. The effects of IHE on intestinal lipid absorption were examined in Wistar rats using a plasma triacylglycerol assay after oral administration of a lipid emulsion. Fecal lipid levels were also measured in these animals after they were fed a high-fat diet containing IHE for 15 days. The effects of IHE on pancreatic lipase activity and the expression of genes involved in hepatic lipid metabolism were also examined using an *in vitro* assay and quantitative RT-PCR, respectively.
RESULTS: Supplementation of high-fat-containing chow with IHE reduced body weight gain and improved glucose tolerance in our experimental mice. A reduction in body weight gain was also observed in C57BL/6N mice fed a standard diet containing IHE. Wistar rats fed a high-fat diet containing IHE showed reduced plasma triacylglycerol levels and an increase in their fecal lipid excretion. Similarly, their pancreatic lipase activity was inhibited and their elevation in plasma triacylglycerol levels seen after the oral administration of lipid emulsion was significantly suppressed. IHE-fed mice showed an increased expression in their lipid oxidation genes and a decreased expression in genes involved in triacylglycerol biosynthesis.
CONCLUSION: The inhibition of intestinal dietary fat absorption may be the mechanism by which IHE induces its weight-lowering effects in high-fat diet-fed mice. The modulatory effect of IHE on lipid metabolism may also, at least partly, be responsible for its beneficial effects on body weight gain. These results suggest that IHE may be helpful in humans in preventing diet-induced obesity and perhaps even metabolic syndrome, the latter of which is known to be associated with obesity.
International Journal of Obesity (2005) **29,** 991–997. doi:10.1038/sj.ijo.0802965; published online 19 April 2005

Keywords: hops; isomerized hop extract; isohumulones; diet-induced obesity

Introduction

Obesity, the abnormal excessive growth of adipose tissue, results from the combined effects of excess energy intake and reduced energy expenditure.[1] The causal association between obesity and the lifestyle-induced metabolic syndrome or syndrome X, which is characterized by the presence of insulin resistance, hypertension, hyperlipidemia, and atherosclerosis, has been recognized for decades.[2] While extensive research has been conducted into the development of antiobesity drugs,[3] the factors in foods derived from plant sources, such as caffeine in oolong tea and capsiate in sweet pepper, for preventing and ameliorating obesity has been investigated.[4–6]

Hops, the female inflorescences of the hop plant (*Humulus lupulus* L.), are used as a preservative and flavoring agent in the beer-brewing process. Humulones, also called α acids, are the primary compounds responsible for imparting the bitter taste to hops and have been shown to suppress the expression of cyclooxygenase-2 (COX-2), a key enzyme that is involved in inflammation and carcinogenesis and which was shown to inhibit angiogenesis.[7] Humulones are

*Correspondence: Dr H Yajima, Central Laboratories for Key Technology, Kirin Brewery Co., Ltd, 1-13-5, Fukuura Kanazawa-ku Yokohama, Kanagawa 236-0004, Japan.
E-mail: hyajima@kirin.co.jp
Received 19 August 2004; revised 15 October 2004; accepted 18 November 2004; published online 19 April 2005

converted, during the beer-brewing process, to isohumulones, also called iso-α acids, which are the actual compounds responsible for imparting the bitter taste to beer. Isohumulones are composed primarily of isohumulone, isocohumulone, and isoadhumulone and exist in beer at concentrations of 20–40 ppm. Isomerized hop extract (IHE), which consists primarily of isohumulones, is made by extracting α acids from the other components in hops, and heating them in an alkaline environment in order to induce isomerization.

The peroxisome proliferator-activated receptors (PPARs) are nuclear fatty acid receptors that have been implicated in obesity-related metabolic diseases such as insulin-resistant type 2 diabetes, hyperlipidemia, and coronary artery disease. Activators of PPARα, such as fibrates, are commonly used to treat hypertriglyceridemia and other dyslipidemic states. Recent data also suggest that activation of PPARα improves insulin resistance that develops as a result of the accumulation of lipid.[8,9] We reported that isohumulones activate PPARs α and γ using *in vitro* reporter assays, and that treatment of diabetic mice with isohumulones decreased their plasma glucose and lipid levels, improved their glucose tolerance, increased their liver fatty acid oxidation, and decreased the size of their hypertrophic adipocytes.[10] In this study, we examined the effects of IHE on obesity and insulin resistance in high-fat diet-fed rodents.

Methods

Isomerized hop extract

IHE (ISOHOPCO2N) was purchased from Botanix Limited (Kent, England). The purity of isohumulones in IHE is 79% and its isohumulone:isocohumulone:isoadhumulone ratio is 37:48:15.

Animals and diets

This study was conducted according to Kirin Pharmaceutical's ethical guidelines for animal care, handling, and termination. Male KK-A^y mice were purchased from Clea Japan (Tokyo, Japan). Female C57BL/6N mice and male Wistar rats were purchased from Charles River Japan (Tokyo, Japan). Animals were maintained under a constant 12-h light–dark cycle (light from 0800 to 2000). Mice were maintained on either a standard (AIN93G[11]) or high-fat (60% of total calories[12]) diet, with or without added IHE. Six C57BL/6N mice (3 mice/cage) on the above *ad libitum* diets had their average energy intake measured daily during the course of the study. Six KK-A^y mice (1 mouse/cage) were fed restricted amounts of diet. Specifically, mice received either 4.5 g of the high fat , or 5.0 g of the normal (AIN), diet daily, with or without IHE. These dietary amounts represent the maximum amount of chow that these animals were found to be able to consume during a 24-h period. Daily caloric intake was 93.3 kJ/day for mice fed the high-fat diet and 78.8 kJ/day for mice fed the AIN diet. Six Wistar rats (1 rat/cage) were similarly fed restricted amounts of these high-fat diets as well.

Measurement of liver triacylglycerol content

To analyze hepatic triacylglycerol levels, approximately 0.2 g of tissue was homogenized and extracted in a chloroform: methanol mixture (2:1 v/v), as previously described.[13] Triacylglycerols were enzymatically quantified using a Triglyceride G Test Wako (Wako Pure Chemicals, Osaka, Japan).

Oral glucose tolerance and insulin tolerance tests

C57BL/6N mice were maintained on equivalent amounts of high-fat diet with or without IHE ($n=6$) for 10 weeks, as described previously,[12] after which time they were subjected to an oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). For oral glucose tolerance testing, D-glucose (1 g/kg body weight) was administered by stomach tube after an overnight fast. Blood samples were collected from the orbital sinus at time 0, and 15, 30, 60, and 120 min after glucose administration, under light anesthesia. For insulin tolerance testing, blood was collected at time 0, after which time human insulin (Humulin R; Eli Lilly, Kobe, Japan) was injected intraperitoneally (0.75 U/kg body weight) into fed mice; tail blood was again collected 15, 30, 60, and 120 min later. Blood glucose levels were determined in these samples using GLUTEST SENSOR (Sanwa Kagaku Kenkyusho Co., Ltd, Nagoya, Japan).

Measurement of fecal lipid content and plasma triacylglycerol levels

Feces of Wistar rats fed a high-fat diet were collected and weighed four times every 24 h from day 12 to day 15. They were then lyophilized and their lipids extracted as previously described,[14] the latter of which were quantified gravimetrically. Tail blood samples were also collected from nonfasted rats on their 14th day on the above dietary regimen, and their triacylglycerol content was determined using Triglyceride G Test Wako (Wako Pure Chemicals, Osaka, Japan).

Measurement of plasma triacylglycerol levels after oral administration of lipid emulsion to Wistar rats

Plasma triacylglycerol levels after oral lipid emulsion were determined as previously described.[15] Briefly, male Wistar rats (8–9 weeks of age) were orally administered 3 ml of lipid emulsion following a 12-h fast. The lipid emulsion consisted of 6 ml of corn oil, 80 mg of cholic acid, 2 g of cholesteryl oleate, and 6 ml of saline. IHE was mixed into the emulsion at a dose of either 50, 100, or 150 mg/kg body weight. Tail blood samples were taken before and 0.5, 1, 2, 3, 4, and 5 h after lipid administration.

Measurement of pancreatic lipase activity *in vitro*

Pancreatic triacylglycerol lipase (EC 3.1.1.3) activity was determined using the LiquiTech™ Lipase Color assay (Roche Diagnostics, Tokyo, Japan). Precinorm™ U (Roche

Diagnostics, Tokyo, Japan) was used as the source of pancreatic triacylglycerol lipase. The effect of IHE on pancreatic triacylglycerol lipase activity was recorded as relative inhibitory activity.

Transient transfection assay

An expression plasmid containing the full-length human PPARα fused to the GAL4 DNA-binding domain was constructed as previously described.[16] The PPARα/GAL4 chimera expression plasmid and pG5 luc plasmid (Promega Corp., Madison, WI, USA) were transfected into HepG2 cells by liposomal delivery using Lipofectamine™ (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. IHE or fenofibrate, a PPARα agonist, was added to the media immediately after transfection. The cells were lysed 48 h later, and their luciferase activity and protein concentration were determined using the Luciferase Assay System (Promega Corp.) and Dc Protein Assay (BIO-RAD, Hercules, CA, USA), respectively; luciferase activity was normalized to protein concentration. Assays were performed in triplicate.

RNA preparation and quantitative RT-PCR

Liver total RNA from KK-A^y mice fed a standard diet with or without IHE for 2 weeks was isolated using ISOGEN (Nippon Gene, Toyama, Japan), and 5 μg of total RNA was utilized for reverse transcription using oligo (dT) primers with the ThermoScript™ RT-PCR system (Invitrogen Corp.). The reverse transcription products were used for quantitative PCR, which was carried out with the LightCycler™ PCR and Detection System using a FastStart DNA Master SYBR Green I kit (Roche Diagnostics GmbH, Mannheim, Germany), as previously described.[17] Relative expression levels of the mRNA of the target genes were normalized to 36B4.[18] To investigate the effects of IHE on fatty acid oxidation in the liver, specific primers for acyl-CoA oxidase (ACO, EC 1.3.3.6) and medium-chain acyl-CoA dehydrogenase (MCAD, EC 1.3.99.3) genes were used. The following specific sense and antisense primers for diacylglycerol *O*-acyltransferase 2 (DGAT2, EC 2.3.1.20) were used to investigate the effects of IHE on triacylglycerol synthesis (GenBank accession numbers are in parentheses): 36B4 (X15267), nucleotides 740–759 and 907–929; ACO (AF006688), nucleotides 1392–1415 and 1607–1628; MCAD (MMU07159), nucleotides 396–417 and 577–596; DGAT2 (NM026384), nucleotides 940–962 and 1125–1147.

Statistical analysis

All values are presented as the mean ± s.d. Differences between groups were evaluated using a nonrepeated measures ANOVA followed by Dunnett's test. Comparison of fecal lipid content, plasma triacylglycerol levels, and gene expression between groups was carried out using Student's *t*-test. Statistical significance was defined as $P<0.05$.

Results

Body/tissue weights and food intake measurements

Body weight gain in C57BL/6N mice fed a high-fat diet containing 0.2 or 0.6% IHE was significantly reduced compared to mice fed a high-fat diet without IHE (control group), in a dose-dependent manner (Figure 1a). Specifically, the body weights of mice fed a diet containing 0.2 or 0.6% IHE were reduced by 14.1 and 22.0%, respectively, compared to the value seen in control animals after 6 weeks of feeding. No significant differences in energy intake were demonstrable between groups (Table 1). Similarly, there were no significant differences in the weight of their heart, spleen, and liver. However, ingestion of IHE prevented the gain in weight in subcutaneous, retroperitoneal, and parametrial adipose tissues and the kidney in a dose-dependent manner (Table 1). Feeding C57BL/6N mice an AIN93G standard diet containing 0.2 or 0.6% IHE for 5 weeks resulted in changes in their body weights such that they were reduced by 9.9 and 13.1%, respectively, compared to the control group (Figure 1b). On the other hand, feeding these mice a high-fat diet containing 0.2 or 0.6% IHE for 5 weeks resulted in body weights that were reduced by 14.0 and 21.3%, respectively, compared to that seen in the control group; note that these reductions were greater than those seen in mice fed standard chow, with the values for the animals fed 0.6% IHE-containing chow reaching statistical significance.

When 6-week-old KK-A^y mice were fed a high-fat diet containing 0.2 and 1.2% IHE for 5 weeks, their body weights were reduced by 9.7 and 10.9% compared to controls, respectively (Figure 1c). A decrease in the weight of subcutaneous and epididymal adipose tissues, as well as the liver and kidney was observed in the IHE diet-supplemented KK-A^y mice (Table 2). The mean weight of the liver and kidney in animals fed chow supplemented with 1.2% IHE was nearly identical to that found in mice fed the AIN93G diet (liver; 2.5 ± 0.25 (g), and kidney; 0.52 ± 0.04 (g)), suggesting that the weight of the liver and kidney in animals fed IHE-containing high-fat diet

Table 1 Tissue weights and energy intake of C57BL/6N mice fed standard (AIN93G) diet or high-fat diet with or without IHE

	AIN control	*HF*	*IHE0.2*	*IHE0.6*
Heart (g)	0.13 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.10 ± 0.01
Spleen (g)	0.076 ± 0.01	0.065 ± 0.01	0.071 ± 0.01	0.065 ± 0.008
Kidney (g)	0.24 ± 0.02	0.27 ± 0.01	0.25 ± 0.02*	0.24 ± 0.03**
Liver (g)	1.1 ± 0.08	1.0 ± 0.1	0.90 ± 0.4	0.95 ± 0.07
Adipose tissue (g)				
Subcutaneous	0.32 ± 0.1	1.9 ± 0.9	1.4 ± 0.4	0.87 ± 0.28**
Retroperitoneal	0.092 ± 0.03	0.44 ± 0.2	0.29 ± 0.1*	0.17 ± 0.06**
Parametrial	0.060 ± 0.03	0.37 ± 0.2	0.20 ± 0.07*	0.13 ± 0.04**
Energy intake (kJ/day)	38.8 ± 1.7	40.6 ± 1.3	38.1 ± 2.9	

IHE was mixed with high-fat diet at 0.2 or 0.6% (w/w). Tissue weights were measured after 6 weeks of feeding. Results are represented as the means ± s.d. of six mice. *$P<0.05$, **$P<0.01$ for high-fat diet-fed group *vs* IHE-supplemented group.

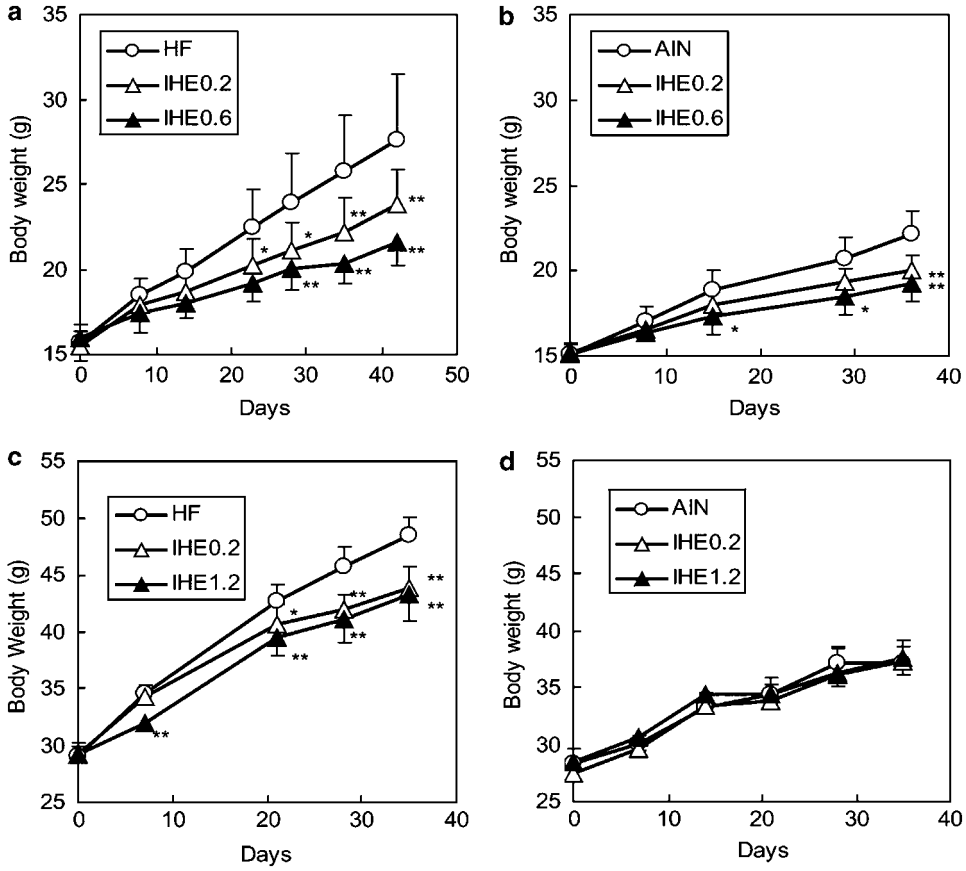

Figure 1 Effects of IHE on body weight in C57BL/6N and KK-A^y mice. Female C57BL/6N mice ($n=6$, 3 mice/cage) were fed a high fat (a) or standard diet (b) that was supplemented with 0.2 or 0.6% (w/w) IHE. Male KK-A^y mice (n = 6, 1 mouse/cage) were fed a high fat (c) or standard diet (d) that was supplemented with either 0.2 or 1.2% IHE. The data represent the mean ± s.d. from 6–8 mice. *$P<0.05$, **$P<0.01$ for the high-fat diet-fed group *vs* the IHE supplemented group on the same day.

Table 2 Tissue weights and liver triacylglycerol content of KK-A^y mice fed high-fat diet with or without IHE

	HF	*IHE0.2*	*IHE1.2*
Liver (g)	3.9 ± 0.3	3.3 ± 0.3**	2.6 ± 0.3**
Liver triacylglycerol (mmol/g liver)	0.21 ± 0.03	0.20 ± 0.05	0.10 ± 0.02**
Kidney (g)	0.72 ± 0.05	0.58 ± 0.06**	0.52 ± 0.06**
Adipose tissue (g)			
Subcutaneous	1.9 ± 0.2	1.4 ± 0.2**	1.4 ± 0.2**
Epididymal	2.0 ± 0.2	2.0 ± 0.2	0.9 ± 0.3

IHE was mixed with diet at 0.2 and 1.2% (w/w). Tissue weight was determined after 5 weeks of feeding. Results are represented as the means ± s.d. of six mice. **$P<0.01$ for high-fat diet-fed group *vs* IHE supplemented group.

was normal. The concentration of triacylglycerols in the liver of IHE diet-supplemented animals was similarly reduced in a dose-dependent manner (Table 2). Administration of IHE to their diet did not affect body weight gain in KK-A^y mice that were fed the normal AIN93G chow (Figure 1d).

Glucose tolerance and plasma insulin levels

After 10 weeks on a high-fat diet containing 0.2 or 0.6% IHE, C57BL/6N mice weighed on average 93.8 and 84.6% of control animals, respectively. After glucose loading, plasma glucose levels in animals treated with IHE were significantly lower than in control mice, suggesting that it improved glucose tolerance (Figure 2a). Although there were no significant differences in plasma glucose levels during the ITT, our results showed a tendency towards reduced insulin resistance in the IHE-supplemented mice (Figure 2b).

Fecal lipid and plasma triacylglycerol levels in Wistar rats

Supplementation of the diet of Wistar rats with IHE significantly increased the lipid content of their feces (64.8 ± 17 mg/g dry feces/day) compared to control rats fed the high-fat diet alone (45.9 ± 11 mg/g dry feces/day). There was no significant difference in the total weight of feces produced by the two groups. Plasma triacylglycerol levels in nonfasted rats that had been on the diet for 14 days were significantly reduced by IHE

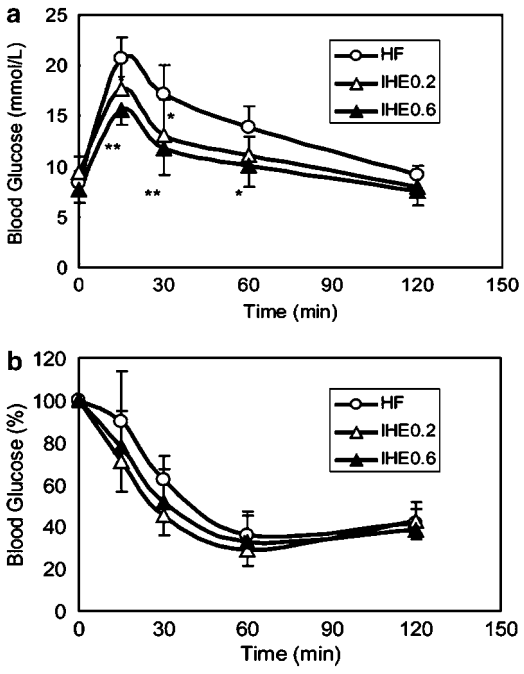

Figure 2 Effects of IHE on glucose tolerance and insulin resistance in mice fed a high-fat diet. C57BL/6N mice were placed on a high-fat diet supplemented with IHE (0.2 or 0.6% (w/w)) for 10 weeks, after which they were subjected to an oral glucose tolerance test (OGTT) (a) and insulin tolerance test (ITT) (b). In the ITT, plasma glucose levels were represented as the percent of the value at time 0. The data represent the mean ± s.d. from six mice. *$P<0.05$, **$P<0.01$ for the high-fat diet-fed group *vs* the IHE supplemented group at each indicated time.

diet supplementation; 100 ± 25 mmol/l for the control and 63.3 ± 19 mmol/l for the IHE-supplemented group, respectively. The body weight gain seen in rats fed the high-fat diet supplemented with IHE was reduced by 9.2% compared to control rats (data not shown).

Plasma triacylglycerol levels after oral administration of lipid emulsion to rats

The serial changes in the plasma triacylglycerol levels of rats after oral administration of lipid emulsion are shown in Figure 3a. Plasma triacylglycerol levels in rats treated with IHE (100 and 150 mg/kg body weight) were significantly reduced compared to rats treated with vehicle at all time points up through 5 h after administration. At 2 h after administration, the elevation in plasma triacylglycerol levels was suppressed by IHE in a dose-dependent manner; peak levels in rats treated with 50, 100, and 150 mg/kg IHE were reduced to 67.9, 36.4, and 20.1%, respectively, of the values seen in control animals.

Effect of IHE on pancreatic triacylglycerol lipase activity

Precinorm™ U, a pancreatic triacylglycerol lipase source, was mixed with various concentrations of IHE and lipase activity

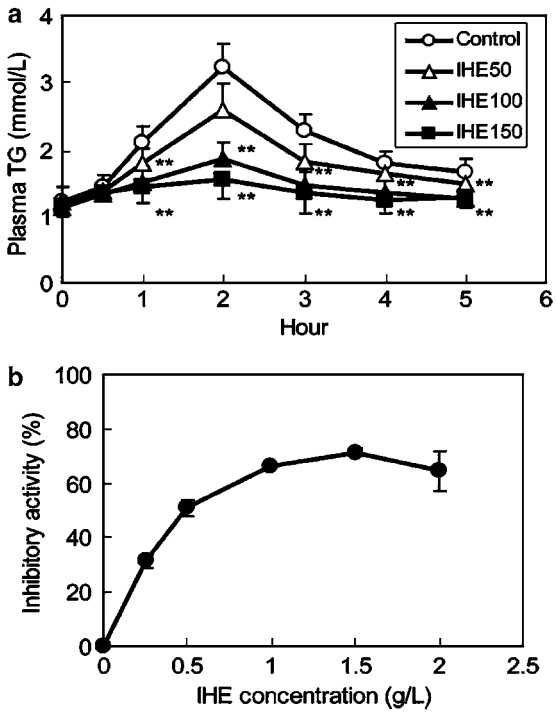

Figure 3 Effects of IHE on intestinal lipid absorption. (a) Wistar rats were orally administered lipid emulsion containing IHE at a concentration of 50, 100, or 150 (mg/kg body weight). Plasma triacylglycerol (TG) levels were quantified at the indicated time after administration of lipid emulsion. The data represent the mean ± s.d. from six rats. **$P<0.01$ for the high-fat diet-fed group *vs* the IHE supplemented group at each indicated time. (b) Effect of IHE on pancreatic lipase activity. The inhibitory effect of IHE on pancreatic lipase was expressed as the relative percent suppression of enzymatic activity. The data represent the mean ± s.d. from three independent experiments.

was determined. As shown in Figure 3b, IHE inhibited pancreatic triacylglycerol lipase activity in a dose-dependent manner; lipase activity was suppressed by about 50% in the presence of 0.5 g/l of IHE.

Effects of IHE on the genes involved in lipid metabolism

A dose-dependent increase in luciferase activity was observed following the addition of IHE to HepG2 cells that were transfected with PPARα and GAL4. The addition of 90 μg/ml of IHE significantly increased luciferase activity compared to the vehicle control (2.53 ± 0.21-fold increase) and this increase was larger than that seen when 1 μM fenofibrate, a specific agonist for PPARα, was added to the cells (1.60 ± 0.15-fold increase). Quantitative RT-PCR analysis of the mRNAs for the acyl-CoA oxidase (ACO), medium-chain acyl-CoA dehydrogenase (MCAD), and diacylglycerol *O*-acyltransferase 2 (DGAT2) genes in the liver of KK-A^y mice fed a standard diet with or without IHE was performed. Our results showed that treatment of mice with IHE significantly increased the mRNA levels of the ACO and MCAD genes while significantly reducing the expression of the DGAT2 gene (Table 3).

Table 3 Effect of IHE on the expression of genes involved in fatty acid metabolism

	Control	*IHE*
Acyl-CoA oxidase	1.00±0.11	1.55±0.15**
Medium-chain acyl-CoA dehydrogenase	1.00±0.21	1.31±0.16*
Diacylglycerol acyltransferase 2	1.00±0.16	0.720±0.068**

Total RNA was isolated from the liver of KK-A^y mice fed a diet with or without 1.2% IHE, which was subjected to quantitative RT-PCR in order to measure the mRNA levels of the indicated genes. Results are expressed as relative expression levels normalized to the expression of the control group (mean±s.d., $n=6$). *$P<0.05$, **$P<0.01$ for the standard diet-fed group (Control) *vs* the IHE-supplemented group (IHE).

Discussion

In this study, we examined the effects of IHE on high-fat diet-induced obesity in C57BL/6N and KK-A^y mice. Our results showed that body weight gain in the groups fed a diet supplemented with IHE was reduced in a dose-dependent manner compared to control animals; this effect was more marked in animals fed a high-fat diet. The fact that there were no differences in food intake in C57BL/6N mice that were fed the various diets suggests that IHE had a direct effect in preventing body weight gain. The body weight-lowering effect of IHE was confirmed in genetically obese KK-A^y mice that were kept on a calorie-restricted diet. It is noteworthy that the body weight-lowering effect of IHE was not observed when KK-A^y mice were fed a regular diet. IHE also improved impaired glucose tolerance, which developed in high-fat diet-fed C57BL/6N mice. Although the results of female C57BL/6N and male KK-A^y mice were shown in this study, the weight-lowering effect of IHE was also observed in male C57BL/6N and female KK-A^y mice (data not shown), showing that the effects of IHE considered to be sex-independent.

Adipose tissue mass in C57BL/6N and KK-A^y mice was significantly reduced by IHE supplementation in a dose-dependent manner. The reduction in kidney weight observed in IHE-supplemented C57BL/6N mice was likely due to the normalization of the kidney hypertrophy that was induced by the high-fat diet, since the kidney weight in mice fed 0.6% IHE was almost the same as that found in mice fed the standard diet. A reduction in the kidney weight was also observed in IHE-supplemented KK-A^y mice, with the kidney weight in the mice fed 0.6% IHE being nearly identical to that seen in mice fed the standard diet. Liver hypertrophy that was observed in KK-A^y mice fed a high-fat diet was prevented by IHE consumption. Hepatic triacylglycerol levels were significantly reduced concomitantly with a decrease in the liver weight in animals treated with IHE. Modulation of lipid metabolism by IHE treatment might have been the cause of the reduction in the liver size and lipid content in these animals (see discussion below).

IHE dose-dependently prevented the rise in plasma triacylglycerol levels seen after the oral administration of lipid emulsion. We also showed that IHE inhibited pancreatic triacylglycerol lipase *in vitro*, which plays a crucial role in intestinal lipid absorption. Furthermore, feeding rats a high-fat diet containing 1% IHE increased their fecal lipid content and decreased their plasma triacylglycerol levels, suggesting that the inhibition of lipase activity by IHE contributed to the reduction in plasma triacylglycerols in these animals. The inhibitory effect of IHE on lipid absorption may have accounted for our data that showed that the reduction in body weight gain induced by IHE in mice fed a high-fat diet was greater than that seen in animals fed a standard diet. It has been reported that teasaponin, an inhibitor of pancreatic lipase activity that was isolated from oolong tea, suppressed the increase in body weight in mice fed a high-fat diet.[19] Accordingly, it is likely that the inhibition of intestinal lipid absorption by IHE was responsible to some extent for IHE's effect in reducing body weight gain.

We previously reported that isohumulone, isocohumulone, and isoadhumulone, which are the main constituents of IHE, activate PPARα and PPARγ. We also revealed that oral administration of isohumulones to obese C57BL/6 mice for 2 weeks resulted in an increase in liver fatty acid oxidation and a normalization of in adipocyte hypertrophy, being likely due to coactivation of PPARα and PPARγ by isohumulones.[10] In the present study, we confirmed that IHE activated PPARα, using an *in vitro* reporter assay. Quantitative RT-PCR analysis of the mRNAs from the liver of KK-A^y mice indicated that the expression of ACO and MCAD, which are known to be regulated by PPARα and to be involved in β-oxidation in liver,[20] was increased by IHE supplementation. In contrast, the expression of the DGAT2 gene, which plays a vital role in the production of triacylglycerol,[21,22] was reduced by IHE. These results suggest that IHE might accelerate lipid oxidation while it, at the same time, suppresses triacylglycerol biosynthesis. Furthermore, in our preliminary study, oral administration of IHE to high-fat diet-fed C57BL/6 mice (330 mg/kg body weight, once a day for 7 days) resulted in significant decrease in body weight (data not shown). Therefore, the modulatory effect of IHE via PPARα activation on lipid metabolism may, at least partly, be responsible for the beneficial effects of IHE on body weight.

Obesity develops when energy intake chronically exceeds energy expenditure, the latter of which occurs as a result of physical activity, adaptive thermogenesis, and basal metabolism.[1] Our results suggest that the beneficial effects of IHE may be mediated by increased lipid oxidation (ie, enhancement of basal metabolism) and by the inhibition of intestinal lipid absorption. We confirmed that the expression level of UCP1, which plays a key role in adaptive thermogenesis[23] in brown adipose tissue of KK-A^y mice, was not affected by IHE treatment (data not shown). Further investigation of the effects of IHE on physical activity and adaptive thermogenesis may provide additional insights into mechanism by which IHE regulates energy metabolism.

In summary, we demonstrated that IHE prevented diet-induced obesity and insulin resistance when included in a high-fat diet, and that these effects may have been mediated by the modulation of lipid metabolism and inhibition of intestinal lipid absorption.

Acknowledgements

We thank K Sato and K Akatsuka for their hands-on assistance with the above experiments.

References

1 Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. *Cell* 2001; **104**: 531–543.
2 Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000; **106**: 473–481.
3 Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; **404**: 661–671.
4 Ohnuki K, Haramizu S, Oki K, Watanabe T, Yazawa S, Fushiki T. Administration of capsiate, a non-pungent capsaicin analog, promotes energy metabolism and suppresses body fat accumulation in mice. *Biosci Biotechnol Biochem* 2001; **65**: 2735–2740.
5 Han LK, Takaku T, Li J, Kimura Y, Okuda H. Anti-obesity action of oolong tea. *Int J Obes Relat Metab Disord* 1999; **23**: 98–105.
6 Birketvedt GS, Travis A, Langbakk B, Florholmen JR. Dietary supplementation with bean extract improves lipid profile in overweight and obese subjects. *Nutrition* 2002; **18**: 729–733.
7 Shimamura M, Hazato T, Ashino H, Yamamoto Y, Iwasaki E, Tobe H, Yamamoto K, Yamamoto S. Inhibition of angiogenesis by humulone, a bitter acid from beer hop. *Biochem Biophys Res Commun* 2001; **289**: 220–224.
8 Ye JM, Doyle PJ, Iglesias MA, Watson DG, Cooney GJ, Kraegen EW. Peroxisome proliferator-activated receptor (PPAR)-alpha activation lowers muscle lipids and improves insulin sensitivity in high fat-fed rats: comparison with PPAR-gamma activation. *Diabetes* 2001; **50**: 411–417.
9 Guerre-Millo M, Gervois P, Raspe E, Madsen L, Poulain P, Derudas B, Herbert JM, Winegar DA, Willson TM, Fruchart JC, Berge RK, Staels B. Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J Biol Chem* 2000; **275**: 16638–16642.
10 Yajima H, Ikeshima E, Shiraki M, Kanaya T, Fujiwara D, Odai H, Tsuboyama-Kasaoka N, Ezaki O, Oikawa S, Kondo K. Isohumulones, bitter acids derived from hops, activate both peroxisome proliferator-activated receptor α and γ and reduce insulin resistance. *J Biol Chem* 2004; **279**: 33456–33462.
11 Reeves PG, Nielsen FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition *ad hoc* writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 1993; **123**: 1939–1951.
12 Ikemoto S, Takahashi M, Tsunoda N, Maruyama K, Itakura H, Ezaki O. High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. *Metabolism* 1996; **45**: 1539–1546.
13 Carr TP, Andresen CJ, Rudel LL. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. *Clin Biochem* 1993; **26**: 39–42.
14 Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* 1957; **226**: 497–509.
15 Han LK, Zheng YN, Xu BJ, Okuda H, Kimura Y. Saponins from *Platycodi radix* ameliorate high fat diet-induced obesity in mice. *J Nutr* 2002; **132**: 2241–2245.
16 Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. *J Biol Chem* 1997; **272**: 3406–3410.
17 Cohen B, Barkan D, Levy Y, Goldberg I, Fridman E, Kopolovic J, Rubinstein M. Leptin induces angiopoietin-2 expression in adipose tissues. *J Biol Chem* 2001; **276**: 7697–7700.
18 Laborda J. 36B4 cDNA used as an estradiol-independent mRNA control is the cDNA for human acidic ribosomal phosphoprotein PO. *Nucleic Acids Res* 1991; **19**: 3998.
19 Han LK, Kimura Y, Kawashima M, Takaku T, Taniyama T, Hayashi T, Zheng YN, Okuda H. Anti-obesity effects in rodents of dietary teasaponin, a lipase inhibitor. *Int J Obes Relat Metab Disord* 2001; **25**: 1459–1464.
20 Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* 1996; **37**: 907–925.
21 Waterman IJ, Zammit VA. Differential effects of fenofibrate or simvastatin treatment of rats on hepatic microsomal overt and latent diacylglycerol acyltransferase activities. *Diabetes* 2002; **51**: 1708–1713.
22 Meegalla RL, Billheimer JT, Cheng D. Concerted elevation of acyl-coenzyme A:diacylglycerol acyltransferase (DGAT) activity through independent stimulation of mRNA expression of DGAT1 and DGAT2 by carbohydrate and insulin. *Biochem Biophys Res Commun* 2002; **298**: 317–323.
23 Nicholls DG, Locke RM. Thermogenic mechanisms in brown fat. *Physiol Rev* 1984; **64**: 1–64.