

## Dietary isohumulones, the bitter components of beer, raise plasma HDL-cholesterol levels and reduce liver cholesterol and triacylglycerol contents similar to PPAR $\alpha$ activations in C57BL/6 mice

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The effects of dietary isohumulones, the main components accounting for the bitter taste of beer, on lipid metabolism were examined. Young female C57BL/6N mice were fed diets containing isomerized hop extract (IHE), which consists mainly of isohumulones. Administration of IHE with an atherogenic (high-fat and high-cholesterol) diet for 2 weeks resulted in a significant increase in plasma HDL-cholesterol ( $P < 0.01$ ), along with a concomitant reduction in the atherosclerosis index, an increase in liver weight and a decrease in body weight gain in a dose-dependent manner. When animals received IHE with either a cholesterol or a basal diet for 1 week, significant decreases in the liver content of cholesterol ( $P < 0.01$ ) and triacylglycerol (cholesterol diet,  $P < 0.01$ ) were observed. Quantitative analyses of hepatic mRNA levels revealed that IHE administration resulted in up-regulation of mRNA for acyl-CoA oxidase, acyl-CoA synthetase, hydroxymethylglutaryl-CoA synthetase, lipoprotein lipase and fatty acid transport protein, and down-regulation of mRNA for Apo CIII and Apo AI. Administration of purified isohumulones effectively resulted in the same changes as IHE. Administration of fenofibrate, an agonist for PPAR $\alpha$ , with a cholesterol diet caused marked hepatomegaly, an increase in plasma HDL-cholesterol, a decrease in hepatic cholesterol content, and alterations in hepatic mRNA levels similar to those observed in mice given IHE. Taken together, these results suggest that the modulation of lipid metabolism observed in mice fed diets containing isohumulones is, at least in part, mediated by activation of PPAR $\alpha$ .

### Isohumulones: Beer: PPAR $\alpha$ : Lipid metabolism

Epidemiological studies have reported that moderate alcohol consumption is associated with decreased risk of CHD. The relationship between the amount of alcohol consumed and risk of CVD has been described as a J-shaped curve: the relative risk of mortality is lower for moderate drinkers than for non-drinkers, but then increases with higher levels of alcohol consumption (Doll *et al.* 1994; Fuchs *et al.* 1995; Gaziano *et al.* 2000). The protective effects of alcohol beverages on the vascular system can be attributed, in part, to the fact that ethanol can increase plasma levels of HDL and reduce thrombotic tendency by inhibiting platelet aggregation (Suh *et al.* 1992).

Non-alcohol components in alcohol beverages are also considered important for their cardioprotective effects. The high antioxidant activity of red wine resulting from the high content of polyphenols is one possible explanation for the 'French paradox' i.e. that some regions of France have lower mortality rates due to CVD than do North European countries, even though the populations are exposed to similar CVD risk factors (dietary fat intake and plasma cholesterol; Renaud & de Lorgeril, 1992; Criqui & Ringel, 1994). Hayek *et al.* (1997) reported that the progression of atherosclerosis in mice deficient in Apo E was reduced by the consumption of red wine or its polyphenols;

this reduction was also associated with a decrease in the susceptibility of LDL to oxidation and aggregation. Furthermore, the consumption of red wine polyphenols in man has been reported to reduce the susceptibility of LDL to oxidation *in vivo* (Nigdikar *et al.* 1998).

Several studies have demonstrated that beer, as well as red wine, has beneficial effects in protecting against CHD (Hoffmeister *et al.* 1999; Brenner *et al.* 2001). It has been reported that moderate consumption of beer does not affect plasma levels of homocysteine, which promotes atherosclerosis and thrombosis, whereas the intake of red wine and spirits increases homocysteine levels (Van der Gaag *et al.* 2000). The high content of folate and vitamin B<sub>6</sub> in beer is likely to be responsible for this effect. This observation is supported by epidemiological studies showing that there is a negative association between beer consumption and plasma homocysteine levels (Mayer *et al.* 2001; Mennen *et al.* 2003).

Beer is the only alcoholic beverage that contains hops, the female inflorescences of the hop plant (*Humulus lupulus* L.). Hops are used as a preservative and flavouring in beer. The bitter taste of beer is mainly attributed to isohumulones, which mostly comprise isohumulone, isochumulone and isoadhumulone. Isohumulones are converted from humulones present in hops by isomerization

**Abbreviations:** 36B4, acidic ribosomal phosphoprotein P0; ACO, acyl-CoA oxidase; ACS, acyl-CoA synthetase; AI, atherosclerosis index; CYP7A1, cholesterol 7 $\alpha$ -hydroxylase; FAS, fatty acid synthetase; FATP, fatty acid transport protein; HMGS, hydroxymethylglutaryl-CoA synthetase; HMGR, hydroxymethylglutaryl-CoA reductase; IHE, isomerized hop extract; LDLR, LDL receptor; LPL, lipoprotein lipase.

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during the wort boiling step of the brewing process. Humulones have been reported to have various biological activities, such as inhibiting angiogenesis, inducing differentiation of myelogenous leukaemia cells and inhibiting bone resorption (Mizobuchi & Sato, 1984, 1985; Miranda *et al.* 1993; Yasukawa *et al.* 1995; Tobe *et al.* 1997*a,b*; Honma *et al.* 1998; Yamamoto *et al.* 2000; Shimamura *et al.* 2001). Although the biological activities of humulones have been studied extensively, there are few studies concerning the activities of the isohumulones found in beer.

Recently, we found from reporter gene expression analyses that isohumulones can activate PPAR  $\alpha$  and  $\gamma$  (Yajima *et al.* 2004). PPAR $\gamma$  activators have been shown to improve insulin sensitivity and are commonly used to treat type 2 diabetes. Dietary administration of isohumulones to diabetic mice resulted in an improvement in insulin resistance, as did pioglitazone, a well-known PPAR $\gamma$  activator (Yajima *et al.* 2004). PPAR $\alpha$  is an important regulator of lipid metabolism and agonists for PPAR $\alpha$ , which stimulate hepatic fatty acid oxidation, are used to treat hyperlipidaemia (Kersten *et al.* 2000). Therefore, it is reasonable to speculate that an intake of isohumulones from beer may modulate lipid metabolism.

The purpose of the present study was to evaluate the effect of isohumulones, the principal components accounting for the bitter taste of beer, on the metabolism of lipids *in vivo* in C57BL/6 mice.

## Materials and methods

### Chemicals

Two types of isomerized hop extract (IHE) containing isohumulones were used. One (Isomerized Kettle Extract; S. S. Steiner, Inc., New York, NY, USA) is a thick brown syrup and contains isohumulones (571 g/l), humulones (10 g/l), lupulones (165 g/l) and hop oil (65 g/l); it is termed IHE1 in this study. The other (ISOHOP CON2; English Hop Products Co. Ltd, Tonbridge, Kent, UK) is an aqueous solution containing isohumulones (300 g/l), and is termed IHE2 in this study. Isohumulones are composed mainly of isohumulone [2-(3-methylbutanoyl)-5-(3-methyl-2-butenyl)-3,4-dihydroxy-4-(4-methyl-3-pentenoyl)-2-cyclopentenone], isocohumulone [2-(2-methylpropanoyl)-5-(3-methyl-2-butenyl)-3,4-dihydroxy-4-(4-methyl-3-pentenoyl)-2-cyclopentenone] and isoadohumulone [2-(2-methylbutanoyl)-5-(3-methyl-2-butenyl)-3,4-dihydroxy-4-(4-methyl-3-pentenoyl)-2-cyclopentenone]. Purified isohumulones were prepared from IHE2 by silica gel chromatography as follows. The IHE2 solution was neutralized to pH 7.8 with HCl and passed over a silica gel column equilibrated with a solution of hexane–ethylacetate (2:1, v/v). Isohumulones were eluted with the same solution and the eluates were evaporated under a stream of N<sub>2</sub> gas. The isohumulones were diluted with an appropriate amount of ethanol and stored at –70°C after being purged with N<sub>2</sub> gas. All reagents and chemicals were extra-pure grade and commercially available.

### Animals

Young female C57BL/6NCRj mice, 5 weeks old, were purchased from Charles River Japan Inc. (Tokyo, Japan). To accustom them to the experimental conditions, the mice were housed in animal rooms for at least 5 d before the experiments, with free access to food and water. Three or four mice were housed per cage, and food intake was measured on a per cage basis. The animals were treated in accordance with Kirin Pharmaceutical's ethical

guidelines for animal care, handling and termination. These guidelines meet the generally accepted international criteria of humane treatment, i.e. sparing the animals needless pain and suffering and ensuring that the experiments conducted are of actual scientific benefit to mankind. At the end of the experiments, the mice were anaesthetized with diethyl ether at about 10.00 hours and killed by draining blood via the postcaval vein. The livers were removed immediately, frozen in liquid N<sub>2</sub> and stored at –80°C.

### Diets and handling of samples

The atherogenic (A) diet was prepared according to Nishina *et al.* (1990, 1993) and contained the following ingredients per kg: 150 g butter, 524.5 g sucrose, 200 g casein, 10 g corn oil, 50 g cellulose, 35 g AIN-93G mineral mixture (Reeves *et al.* 1993), 10 g AIN-93 vitamin mixture (Reeves *et al.* 1993), 2.5 g choline chloride, 3 g cystine, 10 g cholesterol and 5 g sodium cholate. AIN76-A diet (American Institute of Nutrition, 1977), which was purchased from Dyets Inc. (Bethlehem, PA, USA), was used as the basal (B) diet. The cholesterol (C) diet was prepared by adding cholesterol (2 g/kg) to the basal diet. In all experiments, the dietary components were mixed, rolled into pellets with distilled water and stored at –20°C until use to minimize the oxidation of isohumulones. Thawed feed was provided to animals daily.

### Experimental design

In Expt 1, mice were fed the atherogenic diet for 1 week and then divided into five groups of nine animals each (except for the basal diet group, which contained six animals). Over the next 2 weeks, the groups were fed the atherogenic diet, the atherogenic diet supplemented with either two different concentrations of IHE1 (A + IHE1 (low), A + IHE1 (high)) or IHE2 (A + IHE2 (high)), or the basal diet. The A + IHE1 (low) diet contained 2 ml IHE1/kg diet (equivalent to 1.1 g isohumulones/kg diet), the A + IHE1 (high) diet contained 5 ml IHE1/kg diet (equivalent to 2.9 g isohumulones/kg diet), and the A + IHE2 diet contained 10 ml IHE2/kg diet (equivalent to 3 g isohumulones/kg diet).

In Expt 2, mice were divided into four groups of five or six animals each. The groups were maintained for 1 week on the basal diet, the basal diet containing IHE2 (B + IHE2 diet; 10 ml IHE2/kg diet, equivalent to 3 g isohumulones/kg diet), the cholesterol diet, or the cholesterol diet containing IHE2 (C + IHE2 diet; 10 ml IHE2/kg diet, equivalent to 3 g isohumulones/kg diet).

In Expt 3, mice were divided into three groups of eight or nine animals each. The groups were maintained for 2 weeks on the cholesterol diet without supplement, the cholesterol diet containing fenofibrate (C + F diet; 0.5 g fenofibrate/kg diet), or the cholesterol diet containing IHE2 (C + IHE2 diet; 10 ml IHE2/kg diet, equivalent to 3 g isohumulones/kg diet).

In Expt 4, mice were divided into three groups of seven or eight animals each. The groups were maintained for 1 week on the cholesterol diet, the cholesterol diet containing IHE2 (C + IHE2; 10 ml IHE2/kg diet, equivalent to 3 g isohumulones/kg diet), or the cholesterol diet containing purified isohumulones (3 g isohumulones/kg diet).

All blood samples were taken from non-fasted mice except for Expt 1, where blood samples were removed from animals after an overnight fast.

### Biochemical analyses

Plasma lipids (total cholesterol, HDL-cholesterol and triacylglycerols) were determined using commercial kits (Cholesterol C-test, HDL cholesterol-test and Triglyceride G-test, respectively; Wako Pure Chemical, Osaka, Japan). To analyse the hepatic lipid content, approximately 0.2 g liver tissue was homogenized in saline and the lipids were extracted with chloroform-methanol mixture (2:1, v/v) as described by Carr *et al.* (1993). Liver cholesterol and triacylglycerols were measured with the kits described above. Gel filtration chromatography was performed using a Superose 6 HR 10/30 column (Amersham Pharmacia Biotech, Uppsala, Sweden) on 150  $\mu$ l pooled plasma (Ha & Barter, 1985). The gel was allowed to equilibrate with 10 mM-PBS containing EDTA (0.1 g/l) and sodium azide (0.1 g/l). Pooled plasma (150  $\mu$ l) was applied to the column and eluted with the same buffer at 4°C at a flow rate of 0.33 ml/min. The eluate was collected in 0.5 ml fractions. Cholesterol was measured in each fraction using the commercial enzymatic kit described above.

The atherosclerosis index (AI) was calculated as follows:

$$\text{AI} = (\text{total cholesterol} - \text{HDL cholesterol}) / \text{HDL cholesterol}.$$

### Real-time quantitative PCR analysis of gene expression

Light cycler analysis with SYBR green I fluorescence (Roche Diagnostics, Basel, Switzerland) was used to determine the relative mRNA levels of the genes involved in lipid metabolism. Oligonucleotide primers were designed using MacVector 6.5 (Teijin System Technology, Tokyo, Japan) for hydroxymethylglutaryl (HMG)-CoA reductase (HMGR; accession no. X07887 and X07888), HMG-CoA synthetase (HMGS; AK004865), LDL receptor (LDLR; NM\_010700), acyl-CoA oxidase (ACO; NM\_015729), acyl-CoA synthetase (ACS; U15977), fatty acid synthetase (FAS; AF127033), Apo AI (NM\_009693), Apo CIII (NM\_023114), lipoprotein lipase (LPL; NM\_008509), cholesterol 7 $\alpha$ -hydroxylase (CYP7A1; L23754), fatty acid transport protein (FATP; AH006966) and acidic ribosomal phosphoprotein (36B4; X15267). The following PCR reaction primers were used: for HMGR, 5'-TTG GCC TCC ATT GAG ATC CG-3' (5' primer) and 5'-GAT CTT GTT GTC GGT GAA C-3' (3' primer); for HMGCS, 5'-CCT TCA GGG GTC TAA AGC TGG AAG-3' (5' primer) and 5'-CAG CCA ATT CTT GGG CAG AGT G-3' (3' primer); for LDLR, 5'-CAT CAA GGA GTG CAA GAC CAA CG-3' (5' primer) and 5'-CAC TTG TAG CTG CCT TCC AGG TTC-3' (3' primer); for ACO, 5'-ATC TAT GAC CAG GTT CAG TCG GGG-3' (5' primer) and 5'-CCA CGC CAG TTC CTT GCT CTT C-3' (3' primer); for ACS, 5'-GGA ACT ACA GGC AAC CCC AAA G-3' (5' primer) and 5'-CTT GAG GTC GTC CAT AAG CAG C-3' (3' primer); for FAS, 5'-TCG CTC ATG GGT GTG GAA GTT C-3' (5' primer) and 5'-TGC TCA GGT TCA GTT GGT TCT GC-3' (3' primer); for Apo AI, 5'-TGT ATG TGG ATG CGG TCA AAG AC-3' (5' primer) and 5'-TCA TCT CCT GTC TCA CCC AAT CTG-3' (3' primer); for Apo CIII, 5'-AGG GCT ACA TGG AAC AAG CCT C-3' (5' primer) and 5'-CGA CTC AAT AGC TGG AGT TGG TTG-3' (3' primer); for LPL, 5'-GTT TGG CTC CAG AGT TTG ACC G-3' (5' primer) and 5'-CAT ACA TTC CCG TTA CCG TCC ATC-3' (3' primer); for CYP7A1, 5'-ACG GGT TGA TTC CAT ACC TGG G-3'

(5' primer) and 5'-TGT GTC CAA ATG CCT TCG CAG-3' (3' primer); for FATP, 5'-TGC TAG TGA TGG ACG AGC TGG-3' (5' primer) and 5'-TCC TGG TAC ATT GAG TTA GGG TCC-3' (3' primer); and for 36B4, 5'-CCA AGC AGA TGC AGC AGA TCC-3' (5' primer) and 5'-CAG CAG CTG GCA CCT TAT TGG-3' (3' primer).

Total RNA was isolated from the liver samples by using ISOGEN (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions, and 2.5  $\mu$ g of total RNA was used for reverse transcription with oligo(dT) primers and the ThermoScript™ RT-PCR system (Gibco BRL, New York, NY, USA). Aliquots of 20  $\mu$ l were used for light cycler analysis under the conditions suggested by the supplier. Data were analysed using Light Cycler Software (Roche Molecular Biochemicals, Mannheim, Germany). Relative mRNA expression levels of the target genes were normalized to those of 36B4.

### Statistical analysis

Results are expressed as mean values and standard deviations of five to nine mice per group. Statistical analysis of the differences among values was carried out by one-way ANOVA in Expts 1, 3 and 4. When ANOVA revealed a statistical significance, Fisher's protected least significant difference test was performed. In Expt 2, the significance of values was analysed by two-way ANOVA. When the interaction was significant (cholesterol  $\times$  isohumulones), Student's *t* test was performed. Differences were considered significant at  $P < 0.05$ . All statistical analyses were performed using StatView (SAS Institute Inc., Cary, NC, USA).

## Results

### Effects of isomerized hop extracts on body weight and plasma cholesterol and triacylglycerols in mice fed the atherogenic diet (Expt 1)

Two different IHE, IHE1 and IHE2, were used in this experiment. IHE1 contains lupulones and humulones in addition to isohumulones, whereas IHE2 contains isohumulones only (see p. 2). Body weight gain during the 2-week experiment and final body weight of the groups fed IHE with the atherogenic diet (containing high concentrations of cholesterol and fat) were significantly lower ( $P < 0.01$ ) than those of the control group fed the atherogenic diet without hop extracts (Table 1). There was no noticeable decrease in total food intake in the groups given IHE. Administration of the atherogenic diet significantly increased ( $P < 0.01$ ) liver weight by 40% compared with the group fed the basal diet. Supplementation of the atherogenic diet with high doses of IHE1 and IHE2 further increased liver weight (by 12% in each group).

There were no detectable differences in plasma levels of the hepatic enzymes glutamic oxaloacetic transaminase and glutamic pyruvic transaminase among the experimental groups (data not shown). Administration of the atherogenic diet decreased plasma HDL-cholesterol significantly and therefore increased the AI ( $P < 0.01$ ), as compared with the group fed the basal diet. Administration of IHE coupled with the atherogenic diet cancelled out this decrease in HDL-cholesterol at a low dose (A + IHE1 (low), 34% increase) and significantly raised plasma HDL-cholesterol at a high dose (A + IHE1 (high) and A + IHE2 (high), 90% and 70% increase, respectively,  $P < 0.01$ ; Table 1). The increase in plasma total cholesterol levels in the groups receiving diets supplemented with IHE was

**Table 1.** Body weight, energy intake, organ weight and plasma lipid content in mice fed the atherogenic diet, the atherogenic diet supplemented with isomerized hop extract (IHE) and the basal diet for 2 weeks†

(Mean values with standard deviations for nine mice per group in A, A + IHE1 (low), A + IHE1 (high) and A + IHE2 (high) diet groups and for five mice in the B diet group)

	A		A + IHE1 (low)		A + IHE1 (high)		A + IHE2 (high)		B	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight gain (g)	1.75	0.75	0.58**	0.79	0.43**	0.65	0.61**	0.74	1.42	0.68
Final body weight (g)	17.98	1.16	16.70**	0.88	16.44**	0.75	17.07**	0.86	17.20	0.49
Food intake (kJ/2 weeks per mouse)	43.1		41.0		43.1		40.5		43.9	
Tissue weight (g/body kg)										
Liver	56.47	4.65	56.34	4.28	63.42**	6.00	63.38**	4.69	40.31**	3.17
Kidney	11.47	0.89	11.32	0.59	10.96	0.62	10.90	0.77	12.74**	0.55
Plasma total cholesterol (mmol/l)	2.42	0.39	2.83*	0.49	3.48**	0.48	3.34**	0.38	2.39	0.25
Plasma HDL-cholesterol (mmol/l)	0.96	0.16	1.29*	0.25	1.83**	0.31	1.63**	0.16	1.32**	0.14
AI	1.52	0.20	1.26	0.59	0.94**	0.42	1.06**	0.31	0.81**	0.10
Plasma TAG (mmol/l)	0.49	0.07	0.44	0.12	0.46	0.14	0.35*	0.08	0.82**	0.15

A, atherogenic diet group; A + IHE1 (low), A diet supplemented with IHE1 (2 ml/kg, equivalent to 1.1 g isohumulones/kg) group; A + IHE1 (high), A diet supplemented with IHE1 (5 ml/kg, equivalent to 2.9 g isohumulones/kg) group; A + IHE2 (high), A diet supplemented with IHE2 (10 ml/kg, equivalent to 3 g isohumulones/kg) group; B, basal diet group; AI, atherogenic index; TAG, triacylglycerols.

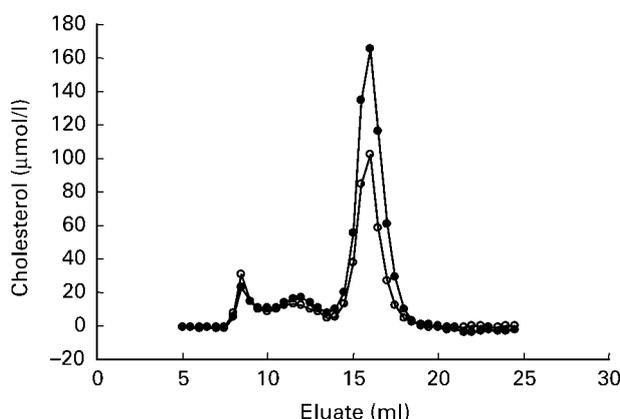
Mean values were significantly different from those of the A diet group: \* $P < 0.05$ , \*\* $P < 0.01$ .

† For details of diets and procedures, see p. 560.

attributed to the increase in HDL-cholesterol. Therefore, the AI values declined in a dose-dependent manner in the groups fed IHE.

Pooled plasma from mice fed the atherogenic diet plus IHE2 (A + IHE2 (high) diet) was fractionated by gel filtration chromatography and the distribution of cholesterol was measured (Fig. 1). The profile suggested that the number of HDL particles in the plasma of IHE-fed mice increased, whereas the number of LDL particles did not change, compared with mice fed the atherogenic diet. This observation is consistent with our result indicating that there was a specific increase in the level of plasma HDL-cholesterol in the groups fed IHE diets (Table 1).

Plasma triacylglycerol levels tended to decrease in the groups given IHE, and this decrease was significant in the group fed the atherogenic diet containing a high dose of IHE2 (A + IHE2 (high), 29% reduction,  $P < 0.05$ ; Table 1). Taken together, these results indicated that the two IHE, IHE1 and IHE2, were equally



**Fig. 1.** Distribution of cholesterol in pooled plasma from mice fed the atherogenic diet (A, ○) and the atherogenic diet supplemented with isomerized hop extract (A + IHE2 (high), ●). Animals were given either the atherogenic diet or the A + IHE2 (high) diet for 2 weeks. In each group, plasma samples of 150 µl were taken from animals whose HDL-cholesterol levels were similar to the mean values of each group. The samples were analysed by gel filtration chromatography using a Superose 6 HR 10/30 column and the eluted fractions were assessed for cholesterol concentration.

effective in changing the parameters investigated (Table 1). Therefore, IHE2, in which the major constituents are isohumulones, was used in the subsequent experiments.

#### *Effect of dietary isomerized hop extract on lipids in the liver (Expt 2)*

We examined the effect of IHE on lipid metabolism by using two diets, the basal diet and the cholesterol diet. A significant suppression in body weight gain was observed in the group fed IHE with the cholesterol diet (C + IHE2, 76% decrease compared with the cholesterol diet,  $P < 0.05$ ), although no significant differences in food intake or final body weight were observed among the four groups (Table 2). Administration of IHE caused an increase in liver weight, regardless of the presence of cholesterol, but this increase was significant only when IHE was given with the basal diet (B + IHE2, 28% increase for the basal diet,  $P < 0.01$ ; Table 2). Dietary IHE tended to increase the level of plasma HDL-cholesterol and thus decrease the AI, although the differences were not so marked as those detected in the groups fed the atherogenic diets. Liver contents of cholesterol and triacylglycerols in mice fed the cholesterol diet plus IHE (C + IHE2) were markedly reduced compared with those found in mice fed the cholesterol diet (38% and 37% decrease, respectively,  $P < 0.01$ ; Table 2). A reduction in liver cholesterol and triacylglycerol contents was also observed in mice fed IHE with the basal diet (B + IHE2, 33% ( $P < 0.01$ ) and 12% decrease, respectively; Table 2).

#### *Effect of dietary isomerized hop extract on hepatic mRNA levels (Expt 2)*

We performed quantitative, real-time, RT-PCR analyses to measure mRNA levels in the livers of mice from four dietary groups (Fig. 2). mRNA levels, which were first normalized to levels of 36B4 mRNA in the same samples as an internal control, were determined relative to the mRNA levels in the group fed the cholesterol diet. Administration of IHE caused an approximately twofold increase in the levels of mRNA for enzymes involved

**Table 2.** Body weight, energy intake, liver weight, plasma lipid content and hepatic lipid content in mice fed the cholesterol diet, the basal diet, and the cholesterol and basal diets supplemented with isomerized hop extract (IHE) for 1 week†  
(Mean values with standard deviations for five or six mice per group)

	C		C + IHE2		B		B + IHE2		ANOVA		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Cholesterol	Isohumulone	Interaction
Body weight gain (g)	1.07	0.41	0.25*	0.42	0.68	0.54	0.82	0.38	NS	NS	0.05
Final body weight (g)	17.09	0.99	16.33	0.40	16.55	0.54	16.60	0.63	NS	NS	NS
Food intake (kJ/week per mouse)	54.0		51.1		54.4		61.5				
Liver weight (g/body kg)	61.92	4.87	67.77	5.47	52.2	1.28	66.61***	3.66	0.01	0.01	0.05
Plasma total cholesterol (mmol/l)	1.90	0.13	2.08	0.14	2.22	0.16	2.11	0.12	0.05	NS	NS
Plasma HDL-cholesterol (mmol/l)	0.98	0.15	1.12	0.05	1.28	0.09	1.33	0.13	0.01	NS	NS
AI	0.98	0.26	0.86	0.15	0.73	0.12	0.59	0.11	0.01	NS	NS
Plasma TAG (mmol/l)	0.81	0.24	0.95	0.19	0.84	0.26	0.71	0.18	NS	NS	NS
Liver lipid ( $\mu$ mol/g liver)											
Cholesterol	30.07	5.40	18.50**	3.60	8.48	1.54	5.65***	0.77	0.01	0.01	0.01
TAG	39.66	8.14	24.97**	3.16	24.97	3.96	21.92	5.76	0.01	0.01	0.05

C, cholesterol diet group; C + IHE2, C diet supplemented with IHE2 (10 ml/kg, equivalent to 3 g isohumulones/kg) group; B, basal diet group; B + IHE2, B diet supplemented with IHE2 (10 ml/kg, equivalent to 3 g isohumulones/kg) group; AI, atherogenic index; TAG, triacylglycerols.

Mean values were significantly different from those of the C diet group: \* $P < 0.05$ , \*\* $P < 0.01$ .

Mean values were significantly different from those of the B diet group: \*\*\* $P < 0.01$ .

† For details of diets and procedures, see p. 560.

in fatty acid oxidation, ACO, ACS, LPL and FATP, regardless of the presence of cholesterol. Administration of IHE also caused a slight but significant increase ( $P < 0.05$ ) in the levels of mRNA for HMGs and LDLR in the basal diet context. By contrast, mRNA levels for apolipoproteins Apo AI and Apo CIII were decreased in the mice given IHE. No significant fluctuation was observed in levels of mRNA for the cholesterologenic enzyme HMGR, the

lipogenic enzyme FAS, and the cholesterol excretion enzyme CYP7A1.

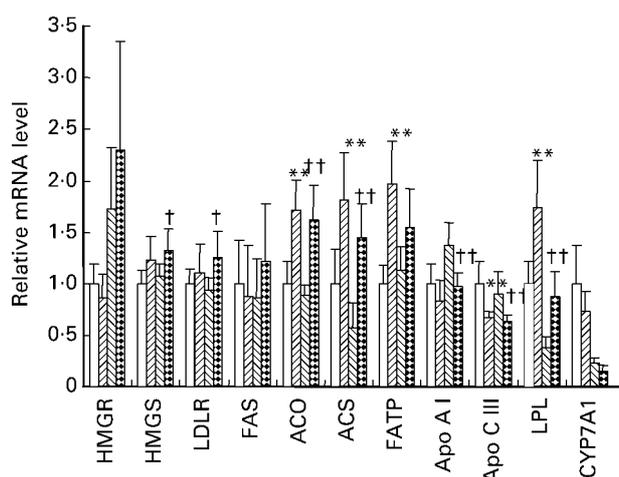
#### Effects of dietary isomerized hop extract and fenofibrate on liver lipid and gene expression (Expt 3)

We compared the effects of IHE on liver lipid content and hepatic mRNA levels with those of fenofibrate, a well-known PPAR $\alpha$  agonist that is used for the medical treatment of hyperlipidaemia. Administration of fenofibrate with the cholesterol diet for 2 weeks exaggerated the changes that had been observed in IHE-fed mice, i.e. marked hepatomegaly (64% increase in the weight), a significant decrease in hepatic cholesterol content (53%), a significant increase in plasma HDL-cholesterol (69%) and a significant decrease in plasma triacylglycerols (56%) were observed in the group given fenofibrate (all  $P < 0.01$ , Table 3). IHE increased liver weight by 19% and decreased hepatic cholesterol by 32% ( $P < 0.01$ ). Administration of IHE also caused a slight increase in HDL-cholesterol and a decrease in plasma triacylglycerols, although the changes were not significant. Notably, IHE caused a greater reduction in hepatic triacylglycerol content than did fenofibrate (33% and 7% reductions, respectively).

The effects of fenofibrate and IHE were assessed by determining the levels of mRNA that are potentially regulated by PPAR $\alpha$  in mice (Fig. 3). Both fenofibrate ( $P < 0.01$ ) and IHE ( $P < 0.05$ ) significantly increased the hepatic levels of mRNA for ACO and LPL, but fenofibrate induced ACO and LPL mRNA levels to a greater extent than did IHE.

#### Comparison of the effects of dietary isomerized hop extract and isohumulones (Expt 4)

Isohumulones were purified from IHE2 and given to the mice for 1 week. The administration of purified isohumulones (3 g/kg cholesterol diet) had almost the same effect as administration of IHE2 (10 ml/kg cholesterol diet, equivalent to 3 g isohumulones/



**Fig. 2.** Quantitative analysis of mRNA in the liver of mice fed the cholesterol diet (C, □), the cholesterol diet supplemented with isomerized hop extract (C + IHE2, ▨), the basal diet (B, ▩) or the basal diet supplemented with IHE2 (B + IHE2, ▤) for 1 week. For each gene, the mRNA level is shown relative to its level in the C group (set as 1.0). Values are means with standard deviations represented by vertical bars for five or six mice per group ( $n = 6$  except for the B group, where  $n = 5$ ). Mean values were significantly different from those of the C group: \* $P < 0.05$ ; \*\* $P < 0.01$ . Mean values were significantly different from those of the B group: † $P < 0.05$ ; †† $P < 0.01$ . ACO, acyl-CoA oxidase; ACS, acyl-CoA synthetase; CYP7A1, cholesterol 7 $\alpha$ -hydroxylase; FAS, fatty acid synthetase; FATP, fatty acid transport protein; HMGR, hydroxymethylglutaryl (HMG)-CoA reductase; HMGs, HMG-CoA synthetase; LDLR, LDL receptor; LPL, lipoprotein lipase.

**Table 3.** Body weight, energy intake, liver weight, plasma lipid content and hepatic lipid content in mice fed the cholesterol diet, and the cholesterol diets supplemented with isomerized hop extract (IHE) and fenofibrate for 2 weeks†

(Mean values with standard deviations for eight or nine mice per group)

	C		C + IHE2		C + F	
	Mean	SD	Mean	SD	Mean	SD
Body weight gain (g)	1.47	0.22	1.12	1.00	2.52	1.05
Final body weight (g)	17.09	0.61	16.52	0.91	17.48	1.23
Food intake (kJ/2 weeks per mouse)	44.4		41.0		43.5	
Liver weight (g/body kg)	55.48	3.12	66.27**	8.47	91.08**	9.26
Plasma total cholesterol (mmol/l)	2.22	0.24	2.27	0.27	3.43**	0.58
Plasma HDL-cholesterol (mmol/l)	1.37	0.22	1.44	0.27	2.31**	0.34
AI	0.63	0.11	0.60	0.13	0.48*	0.12
Plasma TAG (mmol/l)	1.07	0.37	0.99	0.31	0.47**	0.05
Liver lipid ( $\mu$ mol/g liver)						
Cholesterol	41.12	2.83	28.01**	3.86	19.28**	1.54
TAG	57.18	8.93	38.31**	11.41	53.56	12.66

C, cholesterol diet group; C + IHE2, C diet supplemented with IHE2 (10 ml/kg, equivalent to 3 g isohumulones/kg) group; C + F, C diet supplemented with fenofibrate (5 g/kg) group; AI, atherogenic index; TAG, triacylglycerols.

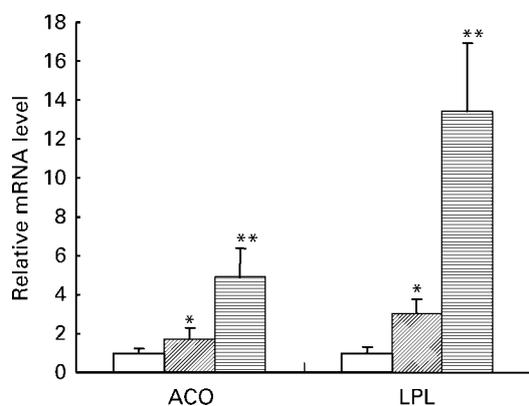
Mean values were significantly different from those of the C diet group: \* $P < 0.05$ , \*\* $P < 0.01$ .

† For details of diets and procedures, see p. 560.

kg cholesterol diet) on liver lipid content and mRNA levels (Table 4 and Fig. 4). No notable difference was observed in the extent of the increase in ACO and LPL mRNA levels or in the extent of the reduction in hepatic cholesterol and triacylglycerol content between the two groups (Table 4 and Fig. 4). From these results, it seems reasonable to conclude that isohumulones are responsible for the hypolipidaemic effects of IHE.

## Discussion

This study was conducted to examine whether isohumulones, the main components accounting for the bitter taste of beer, affect lipid metabolism *in vivo* by using an animal model. Administration of the atherogenic diet containing IHE, in which isohumulones are major ingredients, led to a significant increase in plasma HDL-cholesterol, coupled with a concomitant reduction in the AI,



**Fig. 3.** Quantitative analysis of mRNA in the liver of mice fed the cholesterol diet (C, □), the cholesterol diet supplemented with isomerized hop extract (C + IHE2, ▨) and the cholesterol diet supplemented with fenofibrate (C + F, ■) for 2 weeks. For each gene, the mRNA level is shown relative to its level in the C group (set as 1). Values are means with standard deviations represented by vertical bars for five mice per group. Mean values were significantly different from those of the C group: \* $P < 0.05$ ; \*\* $P < 0.01$ . ACO, acyl-CoA oxidase; LPL, lipoprotein lipase.

in a dose-dependent manner in mice (Table 1). A specific increment in the HDL-cholesterol fraction in plasma was confirmed by gel filtration chromatography (Fig. 1). It should be noted that the significant increase in plasma HDL-cholesterol by IHE was observed on the atherogenic diet (Table 1), while the increases in plasma HDL-cholesterol by IHE on the basal and cholesterol diets were not significant (Tables 2 and 3). A significant decrease in the hepatic triacylglycerol and cholesterol content in mice given cholesterol and basal diets supplemented with IHE was also observed (Tables 2 and 3).

Quantitative analyses of the mRNA in liver revealed that administration of IHE with either the cholesterol or the basal diet caused a significant increase in, or tended to increase, the levels of ACO, ACS, FATP, LPL and HMGs mRNA, and also caused a significant decrease in, or tended to decrease, the levels of Apo AI and Apo CIII mRNA (Fig. 2). The enzymes encoded by ACS, FATP and ACO mRNA are involved in cellular fatty acids uptake, first step prior to transport of fatty acids into the mitochondria, and the peroxisomal  $\beta$ -oxidation of fatty acids, respectively (Kersten *et al.* 2000). An up-regulation of these genes by IHE would therefore increase  $\beta$ -oxidation activity in the liver and would result in a reduction in hepatic triacylglycerol content. Apo CIII and LPL play crucial roles in triacylglycerol metabolism, with early studies having demonstrated that Apo CIII inhibits the hydrolysis of VLDL triacylglycerols by LPL (Sehayek & Eisenberg, 1991; Aalto-setälä *et al.* 1992). The decrease in Apo CIII mRNA and the increase in LPL mRNA in the liver of IHE-fed mice may also be responsible for the reduction in hepatic triacylglycerol content and plasma triacylglycerol levels. Administration of IHE was observed to cause a significant reduction in plasma triacylglycerol levels only in Expt 1 (Table 1, A + IHE2 (high) diet). This may be due to the timing of blood collection (blood was taken from non-fasted mice in Expts 2, 3 and 4). Although a significant decrease in hepatic cholesterol content was observed in mice given IHE (Tables 2, 3 and 4), there was no alteration in the hepatic mRNA levels of HMGs and CYP7A1, which are responsible for the synthesis and excretion of cholesterol, respectively.

**Table 4.** Body weight, liver weight and hepatic lipid content in mice fed the cholesterol diet, and the cholesterol diets supplemented with isomerized hop extract (IHE) and purified isohumulones for 1 week†

(Mean values with standard deviations for seven or eight mice per group)

	C		C + IHE2		C + purified isohumulones	
	Mean	SD	Mean	SD	Mean	SD
Body weight gain (g)	0.80	0.75	-0.40**	0.70	-0.40**	0.73
Final body weight (g)	17.45	1.19	16.22*	0.83	16.50	0.64
Liver weight (g/body kg)	64.39	6.38	69.71*	4.91	67.54	2.96
Liver lipid ( $\mu\text{mol/g}$ liver)						
Cholesterol	25.52	2.26	19.94**	2.29	21.38**	2.54
TAG	41.29	11.38	33.70	8.94	34.33	8.67

C, cholesterol diet group; C + IHE2, C diet supplemented with IHE2 (10 ml/kg, equivalent to 3 g isohumulones/kg) group; C + purified isohumulones, C diet supplemented with purified isohumulones (3 g/kg) group; AI, atherogenic index; TAG, triacylglycerols.

Mean values were significantly different from those of the C diet group: \* $P < 0.05$ , \*\* $P < 0.01$ .

† For details of diets and procedures, see p. 560.

Notably, no difference was detected in the content of cholesterol and bile acid in faeces between the control and IHE-fed mice (data not shown).

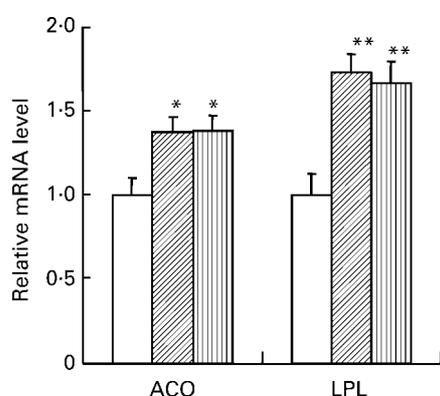
The genes encoding ACO, ACS, FATP and LPL, whose mRNA were up-regulated by the administration of IHE, are known to be regulated by PPAR $\alpha$ , and our experiments using a transient co-transfection assay in mammalian cells have indicated that isohumulones activate PPAR $\alpha$  at a physiological concentration of 10  $\mu\text{M}$  (Yajima *et al.* 2004). We therefore compared the effects of IHE on lipid metabolism with those of fenofibrate, a well-known PPAR $\alpha$  agonist. It has been shown that agonists of PPAR $\alpha$  induce significant hepatomegaly in mice and accelerate the  $\beta$ -oxidation of fatty acids in mitochondria, peroxisomes and microsomes in liver (Reddy *et al.* 1986; Schoonjans *et al.* 1996a,b; Kersten *et al.* 2000; Fruchart, 2001). In the present study, we confirmed that the administration of fenofibrate induced hepatomegaly, reduced hepatic cholesterol content, and increased plasma HDL-cholesterol with a concomitant increase in total cholesterol and a decrease in the AI (Table 3). These changes were similar to those observed in mice fed diets containing IHE, although the significant decrease in hepatic

triacylglycerol content was observed only in the IHE-fed mice and the significant increase in plasma HDL-cholesterol was observed only in fenofibrate-fed mice.

Although fenofibrate has been consistently reported to increase HDL-cholesterol levels in humans (Fruchart, 2001), the results from animal studies are controversial. One study has reported that fenofibrate decreased plasma HDL-cholesterol concentration in a rodent model (Berthou *et al.* 1996). By contrast, fenofibrate has been found to increase HD-cholesterol levels in other rodent models (Oliver *et al.* 1988; Wang *et al.* 2001). It has also suggested that dietary fat content may affect lipoprotein metabolism in animals treated with fibrate-like compounds (Guo *et al.* 2001, Wang *et al.* 2001). In our experimental conditions, administration of the cholesterol diets containing fenofibrate resulted in the significant increase in plasma HDL-cholesterol (Table 3). Notable increase in HDL-cholesterol by IHE could not be detected in the same condition, although the administration of IHE and the atherogenic diet caused a significant increase in plasma HDL-cholesterol (Table 1). This may be due to the difference in lipid and cholesterol contents between the atherogenic and cholesterol diets.

The decrease in Apo AI mRNA levels observed in mice given IHE was inconsistent with the tendency towards an increase in HDL-cholesterol (Fig. 2 and Table 2). The reason for this discrepancy is unclear. Notably, the level of Apo AI mRNA in the liver of fenofibrate-fed mice was also decreased (data not shown). ACO and LPL mRNA in the liver of fenofibrate-fed mice were up-regulated, as were those in mice fed IHE or isohumulones (Figs. 3 and 4). Taken together, these results suggest that the changes in lipid metabolism observed in the mice given IHE are due, at least in part, to the activation of hepatic PPAR $\alpha$  by isohumulones. Fenofibrate has been reported to reduce the hepatic cholesterol content in rodents (Petit *et al.* 1988). The decrease in hepatic cholesterol content observed in IHE-fed mice may be caused by a similar mechanism. Recently, fenofibrate was shown to inhibit a high-fat diet-induced body weight gain in C57BL/6 mice (Guerre-Millo *et al.* 2000), which is compatible with the reduction in body weight observed in mice fed the atherogenic diet with IHE (Table 1).

The present study indicates that IHE i.e. isohumulones and fenofibrate have similar modulatory effects on both plasma and hepatic lipid levels and on the expression of genes involved in lipid metabolism. Our results suggest that activation of the



**Fig. 4.** Quantitative analysis of mRNA in the liver of mice fed the cholesterol diet (C, □), the cholesterol diet supplemented with isomerized hop extract (C + IHE2, ▨) and the cholesterol diet supplemented with purified isohumulones (C + purified isohumulones, ▤) for 1 week. For each gene, the mRNA level is shown relative to its level in the C group (set as 1.0). Values are means with standard deviations represented by vertical bars for six mice per group. Mean values were significantly different from those of the C group: \* $P < 0.05$ ; \*\* $P < 0.01$ . ACO, acyl-CoA oxidase; LPL, lipoprotein lipase.

nuclear receptor PPAR $\alpha$  in liver by isohumulones is at least partly responsible for the effects observed in the mice given IHE. However, the increases in mRNA levels of PPAR $\alpha$ -regulated genes were smaller in the mice given IHE than in those given fenofibrate, although a significant effect on the hepatic lipid content was observed in both groups (Fig. 3). It should be noted that the administration of a low amount of IHE with the cholesterol diet (equivalent to 0.9 g isohumulones/kg cholesterol diet) significantly decreased hepatic cholesterol and triacylglycerol content in mice, without causing a notable increase in ACO and LPL mRNA (data not shown). This result may suggest that the modulatory effects of isohumulones on lipid metabolism are only partially mediated by PPAR $\alpha$ . The amounts of isohumulones taken by the animals fed the diet containing isohumulones at 3 g/kg diet, the most effective concentration, was approximately 500 mg/kg body weight (10 mg per animal). Therefore, the amount of beer could be roughly calculated to be 5–50 l/kg body weight per d, since the concentration of isohumulones in beer varies between 10 and 100 mg/l. It is not realistic to take sufficient amounts of isohumulones through beer to expect the modulatory effect on lipid metabolism. On the basis of our current findings, it is likely that the compounds responsible for beer bitterness, isohumulones, function to normalize lipid metabolism. Further studies are necessary to verify how isohumulones affect lipid metabolism and to assess their potential application as therapeutic agents.

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### References

- Aalto-Setälä K, Fisher EA, Chen X, Chajek-Shaul T, Hayek T, Zechner R, Walsh A, Ramakrishnan R, Ginsberg HN & Breslow JL (1992) Mechanism of hypertriglyceridemia in human apolipoprotein (Apo) CIII transgenic mice. Diminished very low density lipoprotein fraction catabolic rate associated with increased Apo CIII and reduced Apo E on the particles. *J Clin Invest* **90**, 1889–1900.
- American Institute of Nutrition (1977) Report of the American Institute of Nutrition ad hoc writing committee on standard diets for nutritional studies. *J Nutr* **107**, 1340–1348.
- Berthou L, Duverger N, Emmanuel F, *et al.* (1996) Opposite regulation of human versus mouse apolipoprotein A-1 by fibrates in human apolipoprotein A-1 transgenic mice. *J Clin Invest* **97**, 2408–2416.
- Brenner H, Rothenbacher D, Bode G, März W, Hoffmeister A & Koenig W (2001) Coronary heart disease risk reduction in a predominantly beer-drinking population. *Epidemiology* **12**, 390–395.
- Carr TP, Andresen CJ & Rudel LL (1993) Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. *Clin Biochem* **26**, 39–42.
- Cripui MH & Ringel BL (1994) Does diet or alcohol explain the French paradox? *Lancet* **344**, 1719–1723.
- Doll R, Peto R, Hall E, Wheatley K & Gray R (1994) Mortality in relation to consumption of alcohol: 13 years' observations on male British doctors. *Br Med J* **309**, 911–918.
- Fruchart JC (2001) Peroxisome proliferator-activated receptor- $\alpha$  activation and high-density lipoprotein metabolism. *Am J Cardiol* **88**, 24N–29N.
- Fuchs CS, Stampfer MJ, Colditz GA, Giovannucci EL, Manson JE & Kawachi I (1995) Alcohol consumption and mortality among women. *N Engl J Med* **322**, 1245–1250.
- Gaziano JM, Gaziano TA & Glynn RJ (2000) Light-to-moderate alcohol consumption and mortality in the Physicians' Health Study. *J Am Coll Cardiol* **35**, 96–105.
- Guerre-Millo M, Gervois P, Raspé E, *et al.* (2000) Peroxisome proliferator-activated receptor  $\alpha$  activators improve insulin sensitivity and reduce adiposity. *J Biol Chem* **275**, 16638–16642.
- Guo Q, Wang PR, Milot DP, Ippolito MC, Hernandez M, Burton CA, Wright SD & Chao Y (2001) Regulation of lipid metabolism and gene expression by fenofibrate in hamsters. *Biochim Biophys Acta* **1533**, 220–232.
- Ha YC & Barter PJ (1985) Rapid separation of plasma lipoprotein by gel permeation chromatography on agarose gel Superose 6B. *J Chromatogr* **341**, 154–159.
- Hayek T, Fuhrman B, Vaya J, Rosenblat M, Belinky P, Coleman R, Elis A & Aviram M (1997) Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. *Atheroscler Thromb Vasc Biol* **17**, 2744–2752.
- Hoffmeister H, Schelp FP, Mensink GB, Dietz E & Bohning DR (1999) The relationship between alcohol consumption, health indicators and mortality in the German population. *Int J Epidemiol* **28**, 1066–1072.
- Honma Y, Tobe H, Makishima M, Yokoyama A & Okabe-Kado J (1998) Induction of differentiation of myelogenous leukemia cells by humulone, a bitter in the hop. *Leuk Res* **22**, 605–610.
- Kersten S, Desvergne B & Wahll W (2000) Roles of PPARs in health and disease. *Nature* **405**, 421–424.
- Mayer O, Simon J & Rosolova H (2001) A population study of the influence of beer consumption on folate and homocysteine concentrations. *Eur J Clin Nutr* **55**, 605–609.
- Mennen LI, de Courcy GP, Guillard JC, Ducros V, Zarebska M, Bertrais S, Favier A, Hercberg S & Galan P (2003) Relation between homocysteine concentrations and the consumption of different types of alcoholic beverages: the French Supplementation with Antioxidant Vitamins and Minerals Study. *Am J Clin Nutr* **78**, 334–338.
- Miranda CL, Stevens JF, Helmrich A, Henderson MC & Rodriguez RJ (1993) Commonly occurring plant flavonoids have estrogenic activity. *Mol Pharmacol* **44**, 37–43.
- Mizobuchi S & Sato Y (1984) A new flavanone with antifungal activity isolated from hops. *Agric Biol Chem* **48**, 2771–2775.
- Mizobuchi S & Sato Y (1985) Antifungal activity of hop bitter resins and related compounds. *Agric Biol Chem* **49**, 399–403.
- Nigdikar SV, Williams NR, Griffin BA & Howard AN (1998) Consumption of red wine polyphenols reduces the susceptibility of LDL to oxidation *in vivo*. *Am J Clin Nutr* **68**, 258–265.
- Nishina PM, Verstuyft J & Painen B (1990) Synthetic low and high fat diets for the study of atherosclerosis in the mouse. *J Lipid Res* **31**, 859–869.
- Nishina PM, Wang J, Toyofuku W, Kuypers FA, Ishida BY & Painen B (1993) Atherosclerosis and plasma and liver lipids in nine inbred strains of mice. *Lipids* **28**, 599–605.
- Oliver P, Plancke MO, Marzin D, Clavey V, Sauzieres J & Fruchart JC (1988) Effects of fenofibrate, gemfibrozil and nicotinic acid on plasma lipoprotein levels in normal and hyperlipidemic mice. *Atherosclerosis* **70**, 107–114.
- Petit D, Bonnefis MT & Infante R (1988) Effects of ciprofibrate and fenofibrate on liver lipids and lipoprotein synthesis in normo- and hyperlipidemic rats. *Atherosclerosis* **74**, 2215–2241.
- Reddy JK, Goel SK, Nemali MR, Carrino JJ, Laffler TG, Reddy MK, Sperbeck SJ, Osumi T, Hashimoto T & Lalwani ND (1986) Transcription regulation of peroxisomal fatty acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase in rat liver by peroxisome proliferators. *Proc Natl Acad Sci USA* **83**, 1747–1751.

- Reeves PG, Nielsen FH & Fahey GC (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing on the reformation of the AIN-76 rodent diet. *J Nutr* **123**, 1939–1951.
- Renaud S & de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **339**, 1523–1526.
- Schoonjans K, Staels B & Auwerx J (1996a) Role of the peroxisome proliferator-activated receptors (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* **37**, 907–925.
- Schoonjans K, Staels B & Auwerx J (1996b) The peroxisome proliferator-activated receptor (PPAR) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta* **1302**, 92–109.
- Sehayek E & Eisenberg S (1991) Mechanisms of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway. *J Biol Chem* **270**, 18259–18267.
- Shimamura M, Hazato T, Ashino H, Yamamoto Y, Iwasaki E, Tobe H, Yamamoto K & Yamamoto S (2001) Inhibition of angiogenesis by humulone, a bitter acid from beer hop. *Biochem Biophys Res Commun* **289**, 220–224.
- Suh I, Shaten BJ, Culter JA & Kuller LH (1992) Alcohol use and mortality from coronary artery disease: the role of high-density lipoprotein cholesterol. The Multiple Risk Factor Intervention Trial Research Group. *Ann Intern Med* **116**, 881–887.
- Tobe H, Kubota M, Yamaguchi M, Kocha T & Aoyagi T (1997a) Apoptosis to HL-60 by humulone. *Biosci Biotechnol Biochem* **61**, 1027–1029.
- Tobe H, Muraki Y, Kitamura K, Komiyama O, Sato Y, Sugioka T, Maruyama HB, Matsuda E & Nagai M (1997b) Bone resorption inhibitors from hop extract. *Biosci Biotechnol Biochem* **61**, 158–159.
- Van der Gaag MS, Ubbink JB, Sillanaukee P, Nikkari S & Hendriks HF (2000) Effect of consumption of red wine, spirits, and beer on serum homocysteine. *Lancet* **355**, 1522.
- Wang PR, Guo Q, Ippolito M, Wu M, Milot D, Ventre J, Doebber T, Wright SD & Chao YS (2001) High fat fed hamster, a unique animal model for treatment of diabetic dyslipidemia with peroxisome proliferator activated receptor  $\alpha$  selective agonist. *Eur J Pharmacol* **427**, 285–293.
- Yajima H, Ikeshima E, Shiraki M, Kanaya T, Fujiwara D, Odai H, Tsuboyama-Kasaoka N, Ezaki O, Oikawa S & Kondo K (2004) Isohumulones, bitter acids derived from hops, activate both peroxisome proliferator-activated receptor  $\alpha$  and  $\gamma$  and reduce insulin resistance. *J Biol Chem* **279**, 33456–33462.
- Yamamoto K, Wang J, Yamamoto S & Tobe H (2000) Suppression of cyclooxygenase-2 gene transcription by humulon of beer hop extract studied with reference to glucocorticoid. *FEBS Lett* **465**, 103–106.
- Yasukawa K, Takeuchi M & Takido M (1995) Humulon, a bitter in the hop, inhibits tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *Oncology* **52**, 156–158.