Reduced adiposity in bitter melon *(Momordica charantia)* fed rats is associated with lower tissue triglyceride and higher plasma catecholamines

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Slower weight gain and less visceral fat had been observed when rats fed a high-fat diet were supplemented with freeze-dried bitter melon (BM) juice; the metabolic consequences and possible mechanism(s) were further explored in the present study. In a 4-week experiment, rats were fed a low-fat (70 g/kg) or a high-fat (300 g/kg) diet with or without BM (7.5 g/kg or 0.75%). BM-supplemented rats had lower energy efficiency, visceral fat mass, plasma glucose and hepatic triacylglycerol, but higher serum free fatty acids and plasma catecholamines. In the second experiment, 7-week BM supplementation in high-fat diet rats led to a lowering of hepatic triacylglycerol ($P<0.05$) and steatosis score ($P<0.05$) similar to those in rats fed a low-fat diet. BM supplementation did not affect serum and hepatic cholesterol. However, plasma epinephrine and serum free fatty acid concentrations were increased ($P<0.05$). In the third experiment, BM(7.5 and 15 g/kg) and 1.5% BM lowered triacylglycerol concentration in red gastrocnemius and tibialis anterior ($P<0.05$) muscle, but a dose–response effect was not observed. These data suggest that chronic BM feeding leads to a general decrease in tissue fat accumulation and that such an effect is mediated in part by enhanced sympathetic activity and lipolysis. BM or its bioactive ingredient(s) could be used as a dietary adjunct in the control of body weight and blood glucose.

Bitter melon: Adiposity: Triacylglycerol: Catecholamines

Diabetes represents a major public health concern, and its prevalence continues to escalate worldwide (Mokdad *et al.* 2003). A large proportion of diabetics do use herbs and other dietary supplements as adjuncts to traditional treatments (Yeh *et al.* 2003). Bitter melon (*Momordica charantia*, BM), as the most popular plant used by diabetics (Marles & Farnsworth, 1995), is widely grown in tropical areas. The efficacy and safety of BM have been the subject of several recent reviews (Raman & Lau, 1996; Shapiro & Gong, 2002; Basch *et al.* 2003; Yeh *et al.* 2003; Grover & Yadav, 2004). Despite the need for more information in randomised controlled trails, BM-induced decreases in blood glucose appear to be a recognised effect, and no serious adverse effects on humans have been reported. Since the blood glucose-lowering effect is demonstrable with a single oral dose, the acute effect has been attributed to the presence of an insulin-like polypeptide in BM (Khanna *et al.* 1981), a decrease in glucose absorption (Meir & Yaniv, 1985) or an increase in hepatic glucose utilisation (Shibib *et al.* 1993). The mechanisms of actions of BM to lower blood glucose remain inconclusive. Longer-term studies, however, reveal additional potential health benefits of BM.

In our recent study (Chen *et al.* 2003), chronic BM supplementation not only improved glucose tolerance and lowered plasma glucose, but also reduced visceral fat mass and energy efficiency (EE) in rats fed a high-fat (HF) diet. As the latter observations are not attributable to decreases in energy intake or apparent fat absorption, some effects on lipid metabolism were suggested.

The effects of BM feeding on plasma lipids in rats have been reported in normal and chemically induced diabetic rats, with mixed results (Singh *et al.* 1989; Platel *et al.* 1993; Jayasooriya *et al.* 2000; Ahmed *et al.* 2001; Virdi *et al.* 2003). Given that the plasma free fatty acid (FFA) concentration was elevated in rats fed the BM-supplemented HF diet (Chen *et al.* 2003), the objectives of the present study were to determine whether a reduced fat content in tissues is a general phenomenon and to begin to search for further evidence of an enhanced lipolysis by monitoring sympathetic activity. Accordingly, the triacylglycerol (TG) contents of major organs involved in insulin resistance, and plasma catecholamines, were determined in a series of three separate experiments.

Materials and methods

Freeze-dried bitter melon juice and experimental diets

Procedures described previously were followed (Chen *et al.* 2003). Unripe BM fresh fruits were purchased from a local market. Seeds were removed and juice was extracted using an electric juicer. After 72 h of freeze-drying, the yield was about 16 g powder per kilogram fresh fruit. The BM powder was kept in airtight containers at $-70°C$ until use.

The formulation of the powder diets was based on the AIN-93G recommendation (American Institute of Nutrition, 1993). The low fat (LF) diet contained 70 g fat/kg diet. The HF diet (300 g fat/kg

diet) was obtained by adding Crisco shortening (Procter & Gamble, Cincinatti, OH, USA), at the expense of cornstarch. The freeze-dried BM juice powder was also added at the expense of cornstarch (Table 1).

Animals

All animal protocols were approved by the Committee on the Use of Live Animals in Teaching and Research at The University of Hong Kong. The procedures were performed in accordance with the *NIH Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1996). Rats were obtained, at 6 weeks of age, from the existing colony in the animal unit of the Medicine Faculty. They were housed individually in rectangular, hanging wire cages in an environmentally controlled room (12 h cycle with lights on at 07.00 h; $22 \pm 2^{\circ}$C). The rats were given free access to water and consumed laboratory food (Lab Diet – The Richmond Standard; PMI Nutrition International Inc., St Louis, MO, USA) until they were assigned to individual groups. The rats were weighed twice a week. Food cup and spillage were weighed daily, and reported intakes were corrected for spillage. At the end of an experiment, rats that had been food-deprived for 8 h were killed by decapitation. Blood was collected; serum and plasma were stored at -70°C. Liver, visceral fat (Chen *et al.* 2003), tibialis anterior and the red medial part of gastrocnemius (Franch *et al.* 2002) were excised, frozen and stored in liquid N_2 until analysis.

Three experiments were conducted. In experiment 1, BM and HF were introduced to the rats at the beginning. In order to assess the impact of BM supplementation on overweight rats, male rats were pre-exposed to an HF diet for 4 weeks in experiment 2. The experiment was also designed to compare the effect of BM supplementation on the HF-diet rats with those switched from an HF to an LF diet. In the last experiment, female rats were used with the assumption that if BM affects body weight, the effect might be better demonstrated in slow-growing adult females.

Experiment 1. Male Sprague–Dawley rats (170–190g, *n* 32) were randomly assigned to one of four dietary groups. They were fed one of the following diets for 4 weeks (Table 1): LF; LF + BM (0·75 %, w/w); HF; or HF + BM (0·75 %, w/w).

Experiment 2. Male Sprague–Dawley rats (170–190g) were fed an LF (*n* 7) or HF (*n* 32) diet. Beginning at week 5, the HF rats were randomly assigned to one of the four diets for 7 weeks (Table 1): HF, HF + 0·75 % BM (HF/HF + BM), LF (HF/LF) and LF + 0·75 % BM (HF/LF + BM).

Experiment 3. Female Sprague–Dawley rats (140–160g) were fed an LF (*n* 5) or HF (*n* 25) diet for 6 weeks. At the beginning of week 7, some HF rats continued to receive the HF diet (HF, *n* 8) while the others were switched to an HF + 0·75 % BM (*n* 9) or HF + 1·5 % BM (*n* 8) diet for 9 weeks.

Measurements

Plasma glucose was assayed immediately using a commercially available glucose oxidase kit (Kit No. 510-A; Sigma Chemical Co., St Louis, MO, USA). Serum FFA level was determined by an enzymatic colorimetric assay (NEFA C test kit; Wako, Osaka, Japan). Serum insulin was determined by ELISA (Mercodia rat insulin 10-1124-01; Mercordia AB, Uppsala, Sweden).

Serum TG was measured enzymatically (GPO-HDAOS method, L-Type TG-H kit; Wako). With this method, free glycerol was removed in a reaction prior to the hydrolysis of TG and colour development. Serum total cholesterol was determined by a cholesterol oxidase-HDAOS method (L-Type CHO-H kit; Wako). Lipids in liver and muscles were first extracted with a mixture of chloroform and methanol (Folch *et al.* 1957). Lipids were dissolved in *tert*-butyl alcohol, and Triton X-100–methanol (1:1,) was added before enzymatic assays for TG and cholesterol, were carried out (Danno *et al.* 1992).

For plasma catecholamines, a portion of the blood was collected in chilled lithium heparin tubes containing 50 μl of a mixture of EDTA and glutathione (4·75 g EDTA and 3·00 g glutathione dissolved in 50 ml double-distilled deionised water and the pH adjusted to 6·8; Forster & Macdonold, 1999). Plasma epinephrine and norepinephrine were measured by reverse-phase liquid chromatography with electrochemical detection. Plasma epinephrine and norepinephrine were extracted with

Table 1. Composition of experimental diets*

	Low-fat diet (LF)		High-fat diet (HF)		
	LF	LF + 0·75 % BM	HF	HF + 0·75 % BM	HF + 1·5 % BM
Casein (g/kg)†	200·0	200·0	255·0	255·0	255·0
Cornstarch (g/kg)†	529·5	522·0	231·5	224·0	216·5
Sucrose (g/kg)†	100·0	100·0	100·0	100·0	100·0
Cellulose (fibre) (g/kg)†	50·0	50·0	50·0	50·0	50·0
Corn oil (g/kg)‡	70·0	70·0	70·0	70·0	70·0
Shortening (g/kg)‡	0	0	230·0	230·0	230·0
Mineral mix (g/kg)†	35·0	35·0	45·0	45·0	45·0
Vitamin mix (g/kg)†	10·0	10·0	13·0	13·0	13·0
Miscellaneous (g/kg)§	5·514	5·514	5·514	5·514	5·514
Bitter melon (BM) (g/kg)‖	0	7·5	0	7·5	15·0
Energy density (MJ/kg)¶	16·44	16·32	21·01	20·88	20·76

* Based on the AIN-93G diet (American Institute of Nutrition, 1993).
† Harlan Teklad (Madison, WI, USA).
‡ Mazola corn oil (CPC Int., Malaysia); Crisco shortening: Partially hydrogenated vegetable shortening from Procter & Gamble Co. (Cincinatti, OH, USA).
§ Choline bitartrate (2·5 g/kg), L-cystine (3·0 g/kg) and *tert*-butylhydroquinone (0·014 g/kg).
‖ Added as freeze-dried bitter melon juice (BM).
¶ Based on Atwater factors: 16·74 MJ/kg for protein and carbohydrates, 37·66 MJ/kg for fat.

acid-washed alumina (type WA-4; Sigma Chemicals) and desorbed with acetic acid buffer. 3,4-Dihydroxybenzylamine hydrobromide was used as an internal standard. Samples were injected into an HPLC system. The mobile phase (200 ml methanol, 9·02 g sodium acetate, 0·372 g disodium EDTA and 100 mg sodium dodecyl sulphate dissolved in 1000 ml deionised water, pH 5·1) was pumped (PM-80, Bioanalytical Systems, West Lafayette, IN, USA) through a 3 μm octadecylsilane column (PHASE-II, Part No. MF 6213, 100 × 3·2 mm; Bioanalytical Systems) at a rate of 0·7 ml/min. A dual glass carbon electrode was connected to an amperometric detector (LC-4C; Bioanalytical Systems). The potential was set at +0·85 V with an Ag/AgCl reference electrode. The detector range was set at 0·1 μA. Peaks were quantified by area (Allchrom software; Alltech Association Inc., Deerfield, IL, USA). The within-assay CV was 3·8 % and 5·6 % for epinephrine and norepinephrine, respectively. The between-assay CV was 9·9 % for epinephrine and 8·1 % for norepinephrine. Recovery was 92·4 % for epinephrine and 91·7 % for norepinephrine.

Liver histology

The right lobe of the liver from each rat was fixed overnight in 10 % buffered formalin solution and embedded in paraffin. Histological sections (4 μm) were prepared and stained with haematoxylin-eosin. Three sections (12 μm apart) from each rat were graded blindly. A score of 0 to 3 was used to describe the extent of steatosis (Hourigan et al. 1999; Friedenberg et al. 2003). A zero score represents normal liver structure in which lipid accumulation in the hepatocytes is not observed. A score of 1 depicts mild lipid infiltration (occurring in <30 % hepatocytes), a score of 2 depicts moderate lipid infiltration (occurring in 30–70 % hepatocytes), whereas a score of 3 indicates severe lipid infiltration (occurring in >70 % of hepatocytes). In addition, the presence or absence of liver cell injury, including inflammation, fibrosis and necrosis, was also noted.

Statistical methods

Data are expressed as means with their standard errors. Analyses were carried out with the Statistical Package for the Social Science (SPSS for Windows, version 10.1; Chicago, IL, USA). In experiment 1, two-way ANOVA was used to examine the effects of dietary fat (two levels: LF and HF), BM (two levels: with and without BM) and their interactions. In experiments 2 and 3, data were analysed by one-way ANOVA followed by post hoc Duncan's multiple range tests to determine treatment effect and compare differences among group means. Univariate analysis was also carried out to control for differences in energy intake. A non-parametric Kruskal–Wallis test was employed to determine the effect of dietary manipulations on steatosis score. When the scores between two groups were compared, the Mann–Whitney test was applied. Correlation analysis was performed to determine the relationship between two variables. Statistical significance was accepted at $P < 0.05$.

Results

Weight gain and energy intake

In experiment 1, significant effects of dietary fat and BM on weight gain and EE were observed (Table 2). Rats fed an HF-diet consumed more energy. However, BM supplementation did not affect the energy intake of rats. The weight gain of rats given BM was 6·9 % and 9·4 % less than that of their respective LF and HF controls. As a result, BM supplementation led to a reduction in EE ($P < 0.05$).

The growth and EE data for rats in experiment 2 have been reported (Chen et al. 2003; marked with ‡ in Table 4). Rats in the HF/HF + BM group gained less weight and had a lower EE than the HF group ($P < 0.05$). The HF/LF or HF/LF + BM rats all gained less weight and had a lower EE (over 7 weeks, $P < 0.05$) than rats fed an LF-diet for the entire study period.

In experiment 3, EE progressively decreased with increase in BM (4·6, 3·3 and 2·8 for HF, HF + 0·75 % BM and HF + 1·5 % BM, respectively). Weight gain for the three groups over a period of 9 weeks was 124, 78 and 67 g respectively.

Glucose, insulin, free fatty acids and visceral fat mass

In experiment 1, the final concentration of plasma glucose was higher in rats fed an HF diet than in rats fed an LF diet (Table 3; $P < 0.05$). BM supplementation normalised plasma glucose ($P < 0.05$). However, serum insulin concentration and insulin resistance index were similar among the four groups.

There were dietary fat and BM effects on serum FFA and visceral fat mass (Table 3; $P < 0.05$). As dietary fat content increased, serum FFA concentration decreased. In contrast, BM supplementation increased serum FFA concentration. As expected, HF feeding elevated visceral fat mass, whereas BM supplementation lowered visceral fat accumulation. Among the rats in experiment 1, visceral fat mass correlated negatively with serum FFA (r −0·61, n 32, P 0·001) but positively with plasma glucose ($r = 0.48$, n 32, $P = 0.006$).

Table 2. Growth and feed intake of male rats after 4 weeks of bitter melon (BM) supplementation (experiment 1) (Male rats given a low-fat diet (LF) LF + 0·75 % BM, high-fat diet (HF) or HF + 0·75 % BM for 4 weeks)

	LF		LF + 0·75 % BM		HF		HF + 0·75 % BM		P value (two-way ANOVA)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fat	BM	Fat × BM
n	8		8		8		8				
Initial body weight (g)	186	2	187	3	184	3	186	3			
Body weight at week 4 (g)	446	6	430	13	492	10	465	6	0·001	0·031	0·584
Weight gain (g/4 weeks)	260	6	242	12	308	8	279	5	0·01	0·011	0·520
Energy intake (MJ/4 weeks)	14·8	0·4	14·5	0·4	15·9	0·6	15·7	0·3	0·015	0·595	0·973
EE (g gain/MJ)	17·6	0·3	16·7	0·5	19·4	0·3	17·8	0·3	0·001	0·001	0·403

Table 3. Hormone and substrate concentrations of male rats after 4 weeks of bitter melon (BM) supplementation (experiment 1) (Male rats given a low-fat diet (LF) LF + 0·75% BM, high-fat diet (HF) or HF + 0·75% BM for 4 weeks)

	LF		LF + 0·75 % BM		HF		HF + 0·75 % BM		P value (two-way ANOVA)		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Fat	BM	Fat × BM
n	8		8		8		8				
Fasting plasma glucose (mmol/l)	6·5	0·2	6·2	0·3	7·1	0·2	6·6	0·2	0·024	0·040	0·772
Serum insulin (pmol/l)	197·9	27·2	175·6	28·7	153·1	26·4	139·0	21·8	0·131	0·492	0·876
Insulin resistance index (10^{-3} pmol insulin × mmol glucose × L^{-2})	1·29	0·18	1·09	0·19	1·09	0·20	0·93	0·16	0·318	0·311	0·911
Visceral fat mass (g)	23·1	1·4	20·8	1·7	32·2	1·6	26·4	1·0	0·001	0·008	0·240
Serum free fatty acids (mmol/l)	0·57	0·1	0·69	0·1	0·42	0·1	0·56	0·1	0·017	0·027	0·810
Serum triacylglyceroe (mmol/l)	1·4	0·2	2·0	0·5	1·5	0·4	1·0	0·2	0·195	0·800	0·109
Serum cholesterol (mmol/l)	2·8	0·3	3·1	0·3	3·1	0·3	2·8	0·2	0·874	0·995	0·194
Liver weight (g)	16·2	0·6	16·0	0·8	18·5	0·8	17·3	0·7	0·023	0·349	0·534
Liver triacylglycerol (μmol/liver)	390·1	62·5	200·9	16·9	629·5	117·6	514·4	38·6	0·001	0·043	0·610
Liver cholesterol (μmol/liver)	96·2	7·9	85·8	5·2	143·3	24·4	121·8	9·4	0·006	0·260	0·692
Plasma epinephrine (nmol/l)	19·5	2·7	23·7	4·1	15·3	1·5	24·4	2·2	0·547	0·026	0·394
Plasma norepinephrine (nmol/l)	7·7	1·0	17·8	2·3	7·6	1·0	11·3	1·4	0·264	0·024	0·287

Plasma catecholamines

In experiment 1, plasma epinephrine and norepinephrine concentrations were increased with BM supplementation (Table 3; $P < 0.05$).

In experiment 2, plasma epinephrine concentration was lower in the HF rats compared with the LF rats (Table 4; $P < 0.05$). However, the plasma epinephrine concentration of the HF/HF + BM rats was 44·8 % higher than that in the HF rats ($P < 0.05$) and was comparable to that of the LF rats. In this experiment, plasma norepinephrine concentration did not differ among groups. Nevertheless, serum FFA was found to correlate positively with both epinephrine (r 0·34, n 39, $P = 0.034$) and norepinephrine (r 0·33, n 39, $P = 0.038$).

Plasma catecholamines were not measured in experiment 3.

Muscle triacylglycerol and cholesterol

In experiment 3, the TG concentration in both red gastrocnemius and tibialis anterior was higher in HF rats than in LF rats (Fig. 1; $P < 0.05$). The addition of BM to the HF-diet lowered TG concentrations in both types of skeletal muscle ($P < 0.05$).

In all experiments, muscle cholesterol concentration was not affected by dietary manipulations (data not shown).

Hepatic triacylglycerol, cholesterol and histopathology

In experiment 1, feeding an HF-diet led to rats with a greater liver weight, but BM had no impact on liver weight (Table 3). Interestingly, there were effects of dietary fat and BM on hepatic TG content (similar results if based on per gram of liver). The accumulation of TG caused by HF feeding was reduced by 18 % upon BM supplementation ($P < 0.05$).

In experiment 2, the HF/LF rats and HF/HF + BM rats had a similar hepatic TG content that was 40·5 % and 36·9 % lower than that of the HF rats (Table 4; $P < 0.05$).

In experiment 3, liver weight was no different among the four groups (data not shown). The hepatic TG content of the LF, HF + 0·75 % BM and HF + 1·5 % BM groups was similar but significantly lower than that of the HF group (Fig. 1).

Steatosis scores on liver sections from the rats of experiments 2 and 3 are shown in Fig. 2. In experiment 2, the HF/LF, HF/LF + BM and HF/HF + BM rats all had steatosis scores that were not different from the LF rats but were lower than the HF rats ($P < 0.05$). In experiment 3, steatosis scores decreased progressively with BM supplementation. Scores from the HF + 1·5 % BM rats were similar to those of the LF rats but were lower than those of the HF rats ($P < 0.05$).

Photomicrographs of liver sections (experiment 2) characterising the different stages of steatosis are shown in Fig. 3. Fig. 3A represents samples with a score of zero. Over half of the rats fed the LF diet received this score. Rats switched to an LF diet (HF/LF) or fed diets supplemented with varying doses of BM had mild (score of 1; Fig. 3B) to moderate (score of 2; Fig. 3C) macro- and microvesicular steatosis. Macro- and microvesicular steatosis, if present, was predominantly located in the periportal area. As expected, fatty liver was observed in most rats fed an HF diet. These rats had moderate to severe (score of 3; Fig. 3D), mixed micro- and macrovesicular steatosis and even foamy degeneration (Fig. 3E) in the liver. There was no dilatation

Table 4. Effects of bitter melon (BM) supplementation or low-fat diet (LF) on hormone and substrate concentrations of male rats previously exposed to a high-fat diet (HF) (experiment 2)*†

	LF		HF/LF		HF/LF + BM		HF		HF/HF + BM	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
n	7		8		8		8		8	
Body weight at wk 11(g)‡	610[a]	22	626[a]	13	620[a]	12	723[c]	16	675[b]	17
Weight gain (g/7 weeks)‡	175[b]	11	146[a]	10	139[a]	8	235[c]	6	195[b]	7
Energy efficiency (g gain/MJ)‡	7.0[b]	0.3	5.9[a]	0.4	5.9[a]	0.6	8.3[c]	0.2	7.1[b]	0.2
Visceral fat weight (g)‡	45.4[a]	4.6	49.9[a]	3.9	46.9[a]	3.3	79.3[c]	4.5	66.9[b]	4.3
Serum free fatty acid (mmol/l)‡	0.73[ab]	0.03	0.93[c]	0.08	0.92[c]	0.05	0.63[a]	0.06	0.82[bc]	0.07
Serum triacylglycerol (mmol/l)	2.2[ab]	0.3	3.1[b]	0.4	2.1[a]	0.4	2.1[a]	0.3	1.6[a]	0.3
Serum cholesterol (mmol/l)	3.7	0.3	3.8	0.3	4.0	0.3	3.7	0.4	3.4	0.3
Liver weight (g)‡	20.3[a]	0.8	22.1[ab]	0.9	21.3[a]	0.8	24.1[b]	1.0	20.9[a]	0.8
Liver triacylglycerol (μmol/liver)	913.8[a]	129.1	756.1[a]	129.2	573.7[a]	62.9	1270.0[b]	139.1	801.1[a]	137.1
Liver cholesterol (μmol/liver)	169.6	21.3	173.4	23.9	142.3	17.2	213.7	22.7	154.2	20.8
Plasma epinephrine (nmol/l)	64.1[b]	4.9	57.8[ab]	6.0	57.1[ab]	4.9	45.5[a]	4.6	65.9[b]	4.6
Plasma norepinephrine (nmol/l)	29.9	2.9	31.6	7.7	33.2	3.9	32.1	3.9	42.0	5.3

* Male rats were given either an LF diet (n 7) or an HF diet (n 32) for 4 weeks. At the beginning of week 5, the HF rats were randomly assigned to one of the following groups and fed the respective diets for an additional 7 weeks: LF fat (HF/LF), LF + 0.75 % BM (HF/LF + BM), HF (HF), and HF + 0.75 % BM (HF/HF + BM).
† Covariance: total energy intake. Means in a row not sharing a superscript letter are significantly different, $P < 0.05$.
‡ Data from Chen *et al.* (2003).

and congestion in the central veins and in the surrounding sinusoids, and no fibrosis and necrosis in any section.

In all experiments, BM supplementation had no effect on serum TG or serum or hepatic cholesterol concentration.

Discussion

Supplementation with BM slows weight gain and improves insulin sensitivity in rats fed an HF diet. The present results extend our previous study to indicate that the chronic consumption of BM leads to a general decrease in the TG content of the major organs. Elevated plasma catecholamines and serum FFA suggest an enhanced sympathetic activity and lipolytic process. These data are consistent with the view that bioactive ingredients in BM possess anti-obesity properties that might be beneficial to body weight and glycaemic control.

In BM rats, a lower adipose tissue mass did not lead to lipid deposition in other major organs. In fact, BM-supplemented rats had less TG in their livers (Tables 3 and 4, Fig. 1) and skeletal muscle (Fig. 1). This generalised reduction in lipid deposition in the major organs suggests interferences of intermediary metabolism by ingredients present in BM. Rats fed an HF diet have dampened fatty acid synthesis (Romsos & Leveille, 1974), and dietary fatty acids in chylomicrons are taken up by peripheral tissues first before arriving at the liver. Thus, the lower visceral fat and hepatic TG content observed in BM-supplemented rats could be attributable to a decrease in peripheral clearance and enhanced utilisation, respectively. As insulin promotes fat deposition by stimulating lipoprotein lipase (Robinson & Speake, 1989), positive correlations between the insulin resistance index and visceral fat mass in experiment 2 (r 0.653, n 39, $P = 0.001$) and experiment 3 (r 0.847, n 30, $P = 0.001$) do not support an effect involving decreased peripheral uptake. However, a suppressive effect on *de novo* fatty acid synthesis could not be ruled out because LF + BM rats had 10 % less visceral fat and 48.5 % less hepatic TG than LF rats (Table 3).

The elevated concentration of plasma catecholamines is an original observation. Plasma norepinephrine is released from sympathetic nerve endings, and plasma epinephrine is secreted by chromaffin cells of the adrenal medulla. A catecholamine-mediated lipolytic process in BM rats is supported by the positive correlations between serum FFA and plasma catecholamines (see Results). As the decline in weight gain is associated with an enhanced sympathetic activity, the impact of BM on metabolism is reminiscent of that of green tea. Catechin in green tea has been implicated in playing a role in promoting thermogenesis via sympathetic activation (Dulloo *et al.* 2000). Although BM is not known to contain catechin, it is rich in α-eleostearic acid (Yuwai *et al.* 1991; Suzuki *et al.* 2001), a conjugated linolenic acid that could give rise to conjugated linoleic acid (Tsuzuki *et al.* 2003). The latter has been shown to enhance sympathetic activity (Ohnuki *et al.* 2001). Although unlikely, the possibility that sympathetic activation is the consequence of a stress response cannot be ruled out. Activation of the hypothalamo-pituitary–adrenal axis will lead to the secretion of glucocorticoid. This hormone is known to antagonise the effects of insulin and promote lipolysis. Because the overall effects of BM supplementation are improved glucose tolerance (Chen *et al.* 2003) and insulin sensitivity (Table 3), an active role for glucocorticoid in mediating the effects of BM is doubtful.

Blood FFA concentration is determined by lipolytic activity as well as by the rate of uptake by tissues for oxidation or re-esterification. Liver is a key site of metabolic integration. The results of experiments 2 and 3 clearly demonstrated the ability of BM supplementation to reverse steatosis and normalise hepatic TG content. In 7 weeks, the hepatic TG level was reduced by 37 % and 40.5 % when rats switched from an HF to an HF + BM or LF diet (experiment 2). In other words, the addition of BM to an HF diet produced effects similar to those of an LF diet. The lowering of hepatic TG is not limited to rats fed HF diets. Rats given a LF diet with (Table 4) or without (Table 3) prior exposure to an HF diet also had a lower hepatic TG upon BM supplementation. This effect is in general agreement with that reported by others who gave BM pulp (Jayasooriya *et al.* 2000) or juice (Ahmed *et al.* 2001) to rats fed an LF diet. The anti-fatty liver effect of BM may be of clinical interest as diseases such as diabetes, alcoholism and hepatitis can cause steatosis.

The lower TG content of the skeletal muscle of BM-supplemented rats is another important and original observation
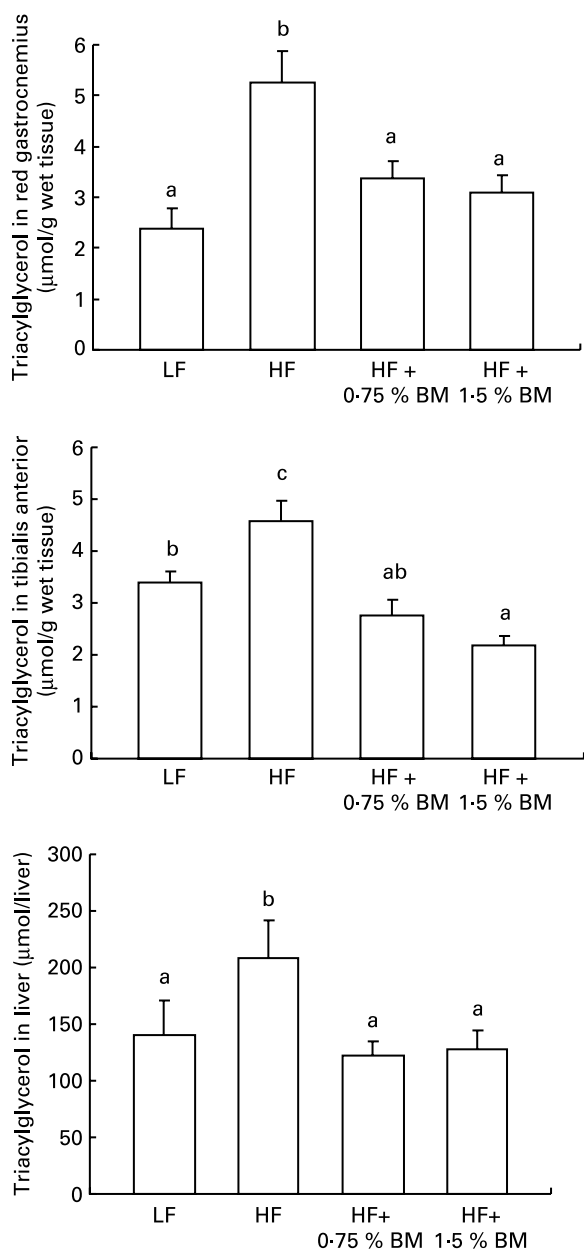
Fig. 1. Triacylglycerol content in the muscle and liver of female rats fed a high-fat diet supplemented with bitter melon (BM) (experiment 3). Rats were given either a low-fat (LF; *n* 5) or a high-fat (HF; *n* 25) diet for 6 weeks. For the following 9 weeks, some of the HF rats were given BM (0·75 % or 1·5 %) supplementation. Values are means with their standard errors represented by vertical bars. (*n* 5–9 rats). Univariate analysis with energy intake as covariate was performed. Mean values not sharing the same letters are significantly different (*P*<0·05; *post hoc* Duncan's multiple range test).

Fig. 2. Histopathological evaluation in the liver of rats. Each section was ranked blindly as described in Materials and methods. In experiment 2 (A), male rats were given either a low-fat (LF) or a high-fat (HF) diet for 4 weeks. At the beginning of week 5, the HF rats were randomly assigned to one of the following groups and fed the respective diets for an additional 7 weeks: low fat (HF/LF), LF + 7.5g/kg BM (HF/LF + BM), HF (HF) and HF + 7.5g/kg BM (HF/HF + BM). In experiment 3 (B), female rats were used (refer to Fig. 1 for experimental conditions). Values are means with their standard errors represented by vertical bars. (*n* 5–9 rats). The overall significance of the steatosis score was determined by the non-parametric Kruskal–Wallis test. *P<0·05 *v.* HF group (Mann–Whitney test).

not previously reported. Skeletal muscle is an important metabolic organ that is responsible for 40–50 % of total FFA disposal (Jensen, 2003). Thus, it is generally considered to be the most important site of insulin resistance (Kelley *et al.* 2002; Hegarty *et al.* 2003). The present results showed that BM supplementation decreased TG content in the oxidative-type (Peters *et al.* 2001) red gastrocnemius and tibialis anterior muscles (experiment 3; Fig. 1). At a dose of 0·75 % BM, TG content was lower than in the unsupplemented HF group and similar to that of the LF group. A dose–response effect was observed for tibialis anterior
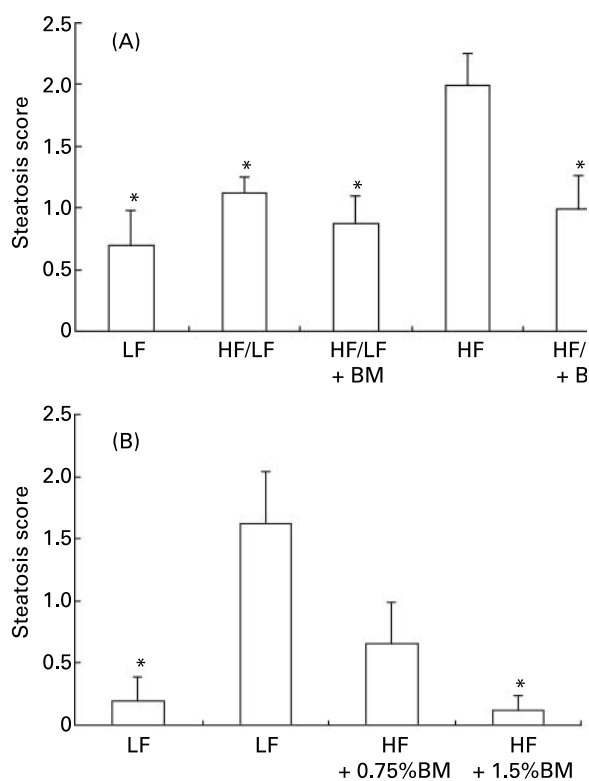
but not for red gastrocnemius. The reduction of TG within muscles may result from a decreased uptake of fatty acids or from an increased capacity for lipid oxidation. The present data suggest that increased lipid oxidation may be responsible, at least in part, for the reduction of muscle TG in BM rats. The significance of the latter phenomenon is further illustrated by a positive correlation between red gastrocnemius TG concentration and insulin resistance index (*r* 0·652, *n* 30, *P*=0·001).

A lack of effect of BM on serum and tissue cholesterol is consistent with that reported by Virdi *et al.* (2003) and Jayasooriya *et al.* (2000) but at variance with that of Platel *et al.* (1993) and Singh *et al.* (1989). The reasons for these discrepant results are not apparent and might be related to factors such as the strain of rat, the presence of diabetes, the BM extract preparation method and the duration of treatment.

The mechanisms whereby BM triggers a series of physiological responses resulting in reduced body fat content and improved insulin sensitivity remain to be elucidated. One consistent finding in our experiments was that of an elevated serum FFA in BM-supplemented rats. Because these rats actually had less tissue TG and better glucose tolerance (Chen *et al.* 2003), the data do not support the general view that FFA induces insulin resistance (Boden *et al.* 2002). It is speculated that, as ligands of peroxisome proliferator-activated receptors, FFA might promote β-oxidation
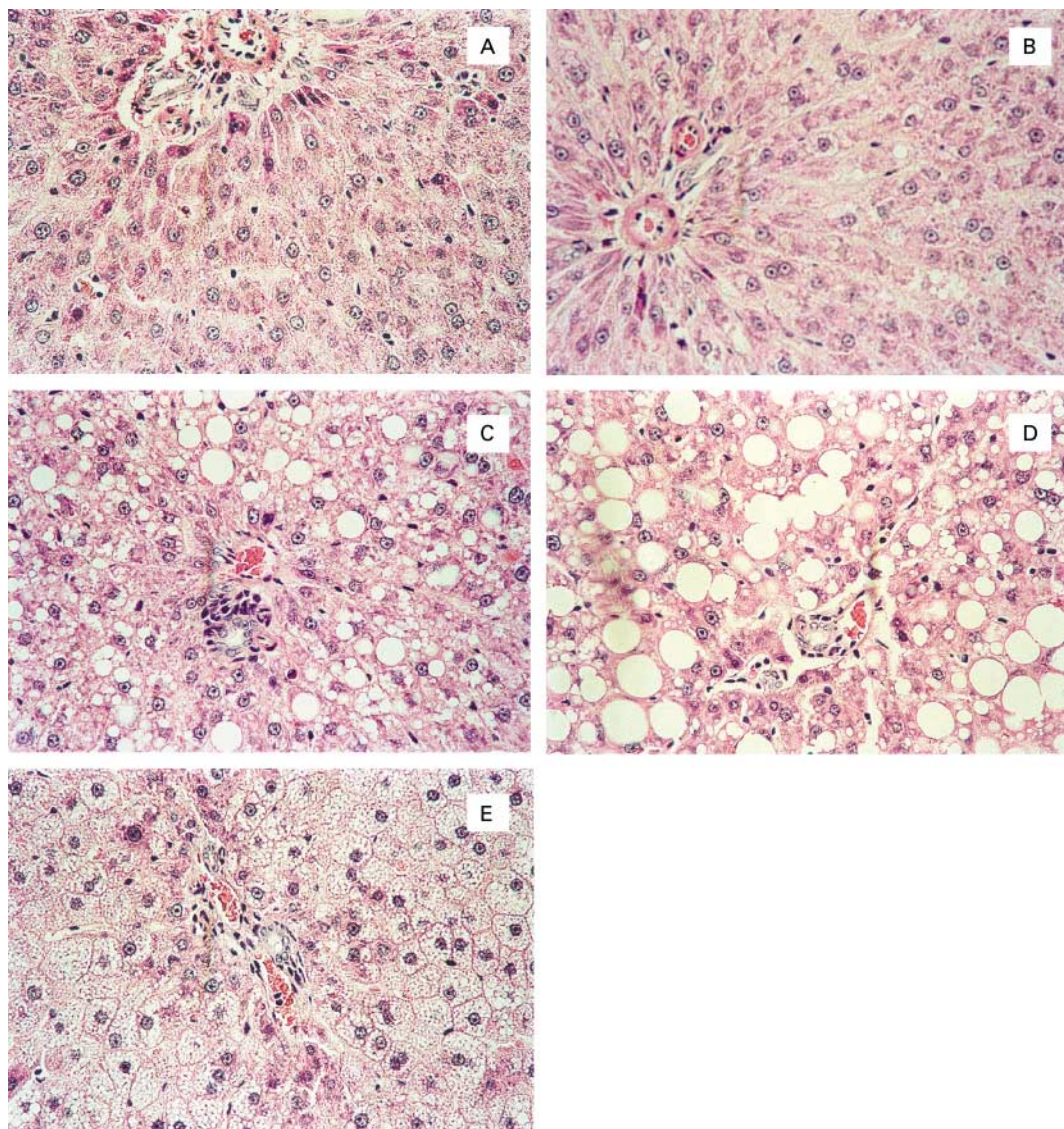
Fig. 3. Representative photomicrographs of liver sections of rats from experiments 2 and 3 (refer to the legends of Figs. 1 and 2 for details). Haematoxylin and eosin stain, original magnification, × 350. (A) normal structure, (B) mild steatosis, (C) moderate steatosis, (D) severe macrovesicular and microvesicular steatosis, and (E) foamy degeneration.

and dissipate energy as heat via an up-regulation of the expression of uncoupling proteins (Armstrong & Towle, 2001). To this end, it is relevant to point out that an ethyl acetate extract of BM (seed or flesh) has recently been shown to activate peroxisome proliferator-activated receptor α and up-regulate the expression of the acyl-CoA oxidase gene in H4IIEC3 hepatoma cells (Chao & Huang, 2003).

In summary, BM supplementation elevates plasma catecholamines and serum FFA level but decreases hepatic and muscular TG content. The data suggest that chronic BM feeding leads to a general decrease in the TG content of the major organs. The latter phenomenon may be mediated in part by enhanced sympathetic activity, lipolysis and possibly lipid oxidation. The positive effects of BM on glycaemia and steatosis warrant further studies to isolate the bioactive ingredients and delineate the mechanisms of actions. In addition to its being a popular vegetable in many countries, the potential benefits of BM might be extended to blood glucose and weight control.

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