

Bitter Melon (*Momordica charantia*) Reduces Adiposity, Lowers Serum Insulin and Normalizes Glucose Tolerance in Rats Fed a High Fat Diet¹

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ABSTRACT Bitter melon (BM) is known for its hypoglycemic effect but its effect on rats fed a hyperinsulinemic high fat diet has not been examined. In a dose-response (0.375, 0.75 and 1.5%) study, oral glucose tolerance was improved in rats fed a high fat (HF; 30%) diet supplemented with freeze-dried BM juice at a dose of 0.75% or higher ($P < 0.05$). At the highest dose, BM-supplemented rats had lower energy efficiency ($P < 0.05$) and tended ($P = 0.10$) to have less visceral fat mass. In a subsequent experiment, rats habitually fed a HF diet either continued to consume the diet or were switched to a HF+BM, low fat (LF; 7%) or LF+BM diet for 7 wk. BM was added at 0.75%. Final body weight and visceral fat mass of the two last-mentioned groups were similar to those of rats fed a LF diet for the entire duration. Rats switched to the HF+BM diet gained less weight and had less visceral fat than those fed the HF diet ($P < 0.05$). The addition of BM did not change apparent fat absorption. BM supplementation to the HF diet improved insulin resistance, lowered serum insulin and leptin but raised serum free fatty acid concentration ($P < 0.05$). This study reveals for the first time that BM reduces adiposity in rats fed a HF diet. BM appears to have multiple influences on glucose and lipid metabolism that strongly counteract the untoward effects of a high fat diet. *J. Nutr.* 133: 1088–1093, 2003.

KEY WORDS: • bitter melon • glucose tolerance • free fatty acids, • visceral fat • hyperinsulinemia • rats

An increase in the prevalence of type 2 diabetes has been reported worldwide (1). Although hypoglycemic agents, dietary fiber and low glycemic index foods are widely available, the public continues to maintain a substantial interest in dietary adjuncts that possess hypoglycemic properties. Over 1000 herbal products have been used by various cultures to lower blood glucose and treat diabetes. Among them, *Momordica charantia* (family Cucurbitaceae, commonly known as ku gua, karela, bittergourd or bitter melon) is the most popular herbal resource (2). Documentation on the pharmacologic properties of bitter melon (BM)³ dates back to the 16th century (3). The hypoglycemic potential of BM has been demonstrated in normal and diabetic rats (4–7) and in patients with type 2 diabetes (5,8). Although BM has been shown to inhibit glucose absorption (9), promote hepatic glucose utilization (6), possess an insulin-like polypeptide (10) and even to increase insulin-positive cell number in the pancreas (11), the mechanism(s) whereby BM lowers blood glucose remain uncertain. Emerging evidence also suggests a hypolipidemic action of BM. BM supplementation lowered serum cholesterol, hepatic total cholesterol and triglyceride in

normal rats (12–14) and elevated HDL cholesterol in streptozotocin-induced diabetic rats (14). With the compelling evidence that BM affects glucose and lipid metabolism, the lack of information in the literature on the effect of BM in rats fed a hyperinsulinemic high fat (HF) diet is surprising. The present study was carried out to test our hypothesis that BM-induced substrate and hormonal changes are associated with a reduction in adiposity. The first experiment examined the dose-dependent effects of freeze-dried BM juice on female rats fed a HF diet. On the basis of improvements in oral glucose tolerance, 0.75%BM was selected for use in the next experiment. The objective of the second experiment was to determine energy efficiency and adiposity of male rats when their habitual HF diet was replaced by a HF+BM, low fat (LF) or LF+BM diet. Potential mechanisms were explored by monitoring apparent fat absorption, serum free fatty acids (FFA), insulin, leptin and tumor necrosis factor- α (TNF- α).

MATERIALS AND METHODS

Preparation of freeze-dried bitter melon juice. Unripe BM fresh fruit purchased from a local market was washed thoroughly with water, cut open and the seeds removed. An electric juicer (Braun MP 80, Kronberg, Germany) was used to extract the juice from the edible portion. The juice (~500 mL/kg) was frozen and then completely lyophilized by continuous freeze-drying operation for 72 h (Dura Bulk Tray Dryer, FTS System, Stone Ridge, NY). The yield was ~16 g powder/kg fresh fruit. The BM powder was kept in airtight containers at -70°C until used.

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³ Abbreviations used: AUC, area under the curve; BM, bitter melon; EE, energy efficiency; FFA, free fatty acids; HF, high fat; IRI, insulin resistance index; LF, low fat; OGTT, oral glucose tolerance test; TNF- α , tumor necrosis factor- α .

Experimental diet. The formulation of the powder diets (Table 1) was based on the AIN-93G recommendation (15). The LF diet contained 7 g fat/100 g diet. The HF diet (30 g fat/100 g diet) was obtained by adding Crisco shortening (Procter & Gamble, Cincinnati, OH), containing mainly partially hydrogenated soybean and cottonseed oils, mono- and diglycerides, at the expense of cornstarch. The freeze-dried bitter melon juice powder was also added at the expense of cornstarch.

Animals. All animal protocols were approved by the committee on the use of live animals in teaching and research, The University of Hong Kong. Rats, at 6 wk of age, were obtained 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 0700 h; $22 \pm 2^\circ\text{C}$). Rats were given free access to water and consumed laboratory food (Lab Diet-The Richmond Standard, PMI Nutrition International, St Louis, MO) until they were assigned to individual groups.

Experiment 1. Female Sprague-Dawley rats were fed a LF or HF diet for 6 wk. At the beginning of wk 7, the LF-fed rats either continued to consume the same diet (LF, $n = 5$) or were switched to a LF diet containing 1.5% BM (LF + 1.5%BM, $n = 4$). Some of the HF rats continued to consume the HF diet (HF, $n = 8$), whereas others were switched to one of the following diets: HF + 0.375%BM ($n = 9$), HF + 0.75%BM ($n = 9$), and HF + 1.5%BM ($n = 8$). Rats were weighed twice weekly. Feed intake was monitored daily. At the end of wk 15, rats that had been food-deprived for 8 h were killed by decapitation between 1600 and 1800 h. Blood was collected from the cervical wound into chilled test tubes. Blood was centrifuged ($2,000 \times g$ at 4°C) and the serum stored at -70°C until used. Liver and visceral (intra-abdominal) fat, including mesenteric, perirenal, perigonadal and retroperitoneal depots, were excised, weighed and stored at -70°C .

Experiment 2. Male Sprague-Dawley rats were fed a LF ($n = 7$) or HF ($n = 32$) diet. Beginning at wk 5, the HF-fed rats were randomly assigned to one of the four diets (Table 1): HF, HF containing 0.75% BM (HF/HF+BM), LF (HF/LF) and LH containing 0.75% BM (HF/LF+BM). At the end of wk 11, the rats were killed; blood and tissues were collected as in the previous experiment. Plasma glucose and serum FFA were determined immediately.

Oral glucose tolerance test (OGTT). The test was conducted on rats after 5 wk of BM supplementation, i.e., in the afternoon of wk 12

and 10 for Experiment 1 and 2, respectively. On test days, rats were deprived of food for 8 h; then a blood sample from the capillary bed of the tail tip was collected in microvette coated with fluoride and heparin (Sarstedt, Germany). Rats then administered a 200 g/L glucose solution by gavage (7.5 mL/kg). Tail blood was collected 30, 60 and 90 min after the gavage.

Apparent fat absorption. In Experiment 2, feces of rats were collected on three consecutive days (d 46–48). The fecal samples were stored at -70°C for the determination of fat content.

Measurements. Plasma glucose was assayed immediately using a commercially available glucose oxidase kit (kit No. 510-A, Sigma Chemical, St. Louis, MO). Serum FFA concentration was determined by an enzymatic colorimetric assay (NEFA C test kit, Wako, Osaka, Japan). ELISA kits were used to measure serum leptin (Active Murine leptin DSL-10–24100, Diagnostic Systems Laboratories, Webster, TX), insulin (Mercodia rat insulin 10–1124-01, Mercodia AB, Uppsala, Sweden) and TNF- α (Rat TNF α Ultra Sensitive KRC 3014, BioSource International, Camarillo, CA). The total fat content in feces and diets was determined gravimetrically. The samples were dried (105°C for 12 h) and then extracted with petroleum ether under reflux (16). On the basis of the 3-d intake and excretion data, apparent fat absorption was calculated using the formula: [(fat intake – fecal fat)/fat intake] $\times 100$.

Statistical methods. Data are expressed as mean \pm SEM. Analyses were carried out with the Statistical Package for the Social Science (SPSS for Windows, version 10.1, Chicago, IL). To determine treatment effect and compare differences among group means, data were analyzed by one-way ANOVA followed by post-hoc Duncan's multiple range test. Correlation analysis was performed to determine the relationship between two variables. Statistical significance was accepted at $P < 0.05$.

An insulin resistance index (IRI) was calculated as the product of insulin and glucose concentration (10^{-3} pmol insulin \times mmol glucose/L²) for each individual rat (17,18). Area under the curve for glucose (AUC_{glucose}) was determined using the trapezoidal rule.

RESULTS

Weight gain and energy intake. In Experiment 1, the 9-wk weight gain of the HF group was 217% that of the LF

TABLE 1

Composition of experimental diets¹

	Low fat diet (LF)			High fat diet (HF)			
	LF	LF + 0.75% BM	LF + 1.5% BM	HF	HF + 0.375% BM	HF + 0.75% BM	HF + 1.5% BM
	<i>g/kg</i>						
Casein ²	200.0	200.0	200.0	255.0	255.0	255.0	255.0
Cornstarch ²	529.5	522.0	514.5	231.5	227.75	224.0	216.5
Sucrose ²	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Cellulose (fiber) ²	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Corn oil ³	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Shortening ⁴	0	0	0	230	230	230	230
Mineral mix ²	35.0	35.0	35.0	45.0	45.0	45.0	45.0
Vitamin mix ²	10.0	10.0	10.0	13.0	13.0	13.0	13.0
Miscellaneous ⁵	5.514	5.514	5.514	5.514	5.514	5.514	5.514
Bitter melon (BM) ⁶	0	7.5	15.0	0	3.75	7.5	15.0
	<i>MJ/kg</i>						
Energy density ⁷	16.44	16.32	16.19	21.01	20.95	20.88	20.76

¹ Based on the AIN-93G diet (15).

² Harlan Teklad (Madison, WI).

³ Mazola (CPC, Malaysia).

⁴ Crisco, partially hydrogenated vegetable shortening from Procter & Gamble (Cincinnati, OH).

⁵ Choline bitartrate (2.5 g/kg), L-cystine (3.0 g/kg) and *tert*-butylhydroquinone (0.014 g/kg).

⁶ Added as freeze-dried bitter melon juice (BM).

⁷ Based on Atwater factors: 16.74 MJ/kg for protein and carbohydrates, and 37.66 MJ/kg for fat.

TABLE 2

Effects of bitter melon (BM) supplementation on growth and feed intake of female rats fed low fat (LF) or high fat (HF) diets (Experiment 1)^{1,2}

	Low fat diet (LF)		High fat diet (HF)			
	LF	LF + 1.5% BM	HF	HF + 0.375% BM	HF + 0.75% BM	HF + 1.5% BM
<i>n</i>	5	4	8	9	9	8
Body weight at wk 7, g	280 ± 6a	280 ± 11a	341 ± 16b	341 ± 15b	340 ± 15b	341 ± 18b
Body weight at wk 15, g	337 ± 15ab	311 ± 16a	465 ± 47c	445 ± 32c	418 ± 20bc	409 ± 29abc
Weight gain, g/9 wk	57 ± 11ab	31 ± 6a	124 ± 32c	103 ± 18bc	78 ± 7abc	67 ± 12abc
Energy intake, MJ/9 wk	22.7 ± 1.4	19.8 ± 0.6	25.0 ± 2.0	24.0 ± 1.2	23.3 ± 0.6	22.8 ± 1.0
Energy efficiency, g gain/MJ	2.4 ± 0.3ab	1.6 ± 0.3a	4.6 ± 0.7d	4.1 ± 0.5cd	3.3 ± 0.2bcd	2.8 ± 0.4abc

¹ Female rats were given either LF (*n* = 9) or HF (*n* = 34) diets for 6 wk before random assignment to their respective groups.

² Results are expressed as means ± SEM. Means in a row not sharing a letter are significantly different, *P* < 0.05.

group (Table 2). Although weight gain of the HF + 1.5%BM group was only 54% that of the HF group, large within-group variability rendered the dose-response effect nonsignificant (*P* = 0.075). With the addition of BM, energy efficiency (EE) progressively decreased. Energy efficiency of the HF + 1.5%BM group was significantly lower than that of the HF group (*P* < 0.05).

In Experiment 2, male rats switched from a HF to a LF diet with or without BM gained less weight (over 7 wk, *P* < 0.05) and had lower EE (*P* < 0.05) than rats fed a LF diet for the entire period (Table 3). Weight gain and EE did not differ between the HF/LF and HF/LF+BM groups. Rats in the HF/HF+BM group gained less weight and had lower EE than the HF group (*P* < 0.05). In Experiment 2, the addition of BM to the HF or LF diet did not affect apparent fat absorption (Table 3).

Visceral fat mass and liver weight. Visceral fat mass of the HF group was higher than that of the LF group (*P* < 0.05). There was a trend (*P* = 0.10) for a decrease in visceral fat mass when female rats were supplemented with BM (Table 4). Fat mass in these rats also correlated with body weight gain (*r* = 0.930, *df* = 41, *P* < 0.01).

A clearer effect of BM on adiposity was observed in male rats fed the HF diet (Table 5). Visceral fat mass of the HF/HF+BM group was lower than that of the HF group (*P* < 0.05). However, there was no difference between the HF/LF and HF/LF+BM groups. Liver weights of the HF/LF+BM and HF/HF+BM groups were similar (Table 5) and were lower than those of the HF group (*P* < 0.05).

Oral glucose tolerance. The addition of BM to the HF diet improved oral glucose tolerance (Fig. 1). The AUC_{glucose} of the HF + 0.75%BM and HF + 1.5%BM groups were lower than that of the HF group (*P* < 0.05).

In Experiment 2, the OGTT yielded similar results among the LF, HF/LF, HF/LF+BM and HF/HF+BM groups (Fig. 2). The AUC_{glucose} of the HF group was higher than that of the other four groups (*P* < 0.05).

Insulin, leptin, TNF-α and FFA. In Experiment 1, serum insulin and leptin correlated strongly with visceral fat mass (*r* = 0.843 and 0.743, *df* = 32, *P* < 0.001) among the HF-fed rats. There was a trend (*P* = 0.059) for IRI to decrease with the addition of BM (Table 4).

In Experiment 2, IRI was significantly lower for the LF, HF/LF, HF/LF+BM and HF/HF+BM rats compared with the HF rats (*P* < 0.005, Table 5). Male rats switched from the HF to the LF, LF+BM or HF+BM diet had significantly lower serum leptin concentration (*P* < 0.05). In this experiment, serum TNF-α concentrations did not differ among groups. Its concentration did not correlate with visceral fat mass (*r* = -0.28, *df* = 37, *P* = 0.088). Interestingly, serum FFA

TABLE 3

Effects of a low fat (LF) diet with or without bitter melon (BM) on body weight, energy efficiency and apparent fat absorption in male rats previously exposed to a high fat (HF) diet (Experiment 2)^{1,2}

	LF	HF/LF	HF/LF+BM	HF	HF/HF+BM
<i>n</i>	7	8	8	8	8
Body weight at wk 5, g	435 ± 13a	480 ± 8b	482 ± 7b	487 ± 11b	481 ± 11b
Body weight at wk 11, g	610 ± 22a	626 ± 13a	620 ± 12a	723 ± 16c	675 ± 17b
Weight gain, g/7 wk	175 ± 11b	146 ± 10a	139 ± 8a	235 ± 6c	195 ± 7b
Energy intake, MJ/7 wk	24.7 ± 0.7a	24.4 ± 0.7a	23.4 ± 0.4a	28.4 ± 0.5b	27.4 ± 0.4b
Energy efficiency, g gain/MJ	7.0 ± 0.3b	5.9 ± 0.4a	5.9 ± 0.6a	8.3 ± 0.2c	7.1 ± 0.2b
Fat intake, ³ g/3 d	6.6 ± 0.3a	6.6 ± 0.2a	6.2 ± 0.2a	24.4 ± 0.6b	23.5 ± 0.3b
Fecal fat, g/3 d	0.26 ± 0.02a	0.30 ± 0.01a	0.30 ± 0.01a	0.92 ± 0.04b	0.84 ± 0.03b
Apparent absorption, %	96.1 ± 0.3b	95.4 ± 0.2a	95.2 ± 0.1a	96.3 ± 0.2b	96.4 ± 0.1b

¹ Male rats were given either a low fat diet (*n* = 7) or a high fat diet (*n* = 32) for 4 wk. At the beginning of wk 5, the HF rats either continued to consume the HF diet or were randomly assigned to one of the following diets: low fat (HF/LF), low fat with 0.75% BM (HF/LF + BM), high fat with 0.75% BM (HF/HF + BM).

² Results are expressed as means ± SEM. Means in a row not sharing a letter are significantly different, *P* < 0.05.

³ Food intake and fecal output were monitored for 3 consecutive days in wk 7.

TABLE 4

Visceral fat mass, plasma glucose, serum insulin and leptin concentrations of rats fed low fat (LF) or high fat (HF) diets after 9 wk of bitter melon (BM) supplementation (Experiment 1)^{1,2}

	Low fat diet (LF)		High fat diet (HF)			
	LF	LF + 1.5% BM	HF	HF + 0.375% BM	HF + 0.75% BM	HF + 1.5% BM
<i>n</i>	5	4	8	9	9	8
Visceral fat pad weight, g	28.9 ± 3.6 ^{ab}	20.9 ± 3.8 ^a	60.0 ± 9.3 ^c	55.6 ± 7.0 ^c	45.5 ± 5.2 ^{bc}	42.1 ± 5.0 ^{abc}
Final plasma glucose, mmol/L	6.1 ± 0.3 ^{ab}	5.2 ± 0.1 ^a	7.6 ± 0.4 ^c	6.8 ± 0.3 ^{bc}	6.2 ± 0.2 ^b	6.0 ± 0.3 ^{ab}
Serum insulin, pmol/L	145.9 ± 28.6 ^{ab}	132.6 ± 19.6 ^a	418.0 ± 94.9 ^c	371.1 ± 83.2 ^{bc}	236.0 ± 32.0 ^{abc}	224.5 ± 58.4 ^{abc}
IRI, 10 ⁻³ pmol insulin × mmol glucose/L ²	0.91 ± 0.20 ^a	0.69 ± 0.10 ^a	3.34 ± 0.64 ^b	2.65 ± 0.49 ^{ab}	1.47 ± 0.22 ^{ab}	1.43 ± 0.42 ^{ab}
Serum leptin, µg/L	1.8 ± 0.7 ^{ab}	1.6 ± 0.7 ^a	5.1 ± 1.1 ^c	4.5 ± 1.0 ^{bc}	3.0 ± 0.5 ^{abc}	2.7 ± 0.6 ^{abc}

¹ Rats that had been deprived of food for 8 h were killed at the end of wk 15.

² Results are expressed as means ± SEM. Means in a row not sharing a letter are significantly different, *P* < 0.05. IRI, insulin resistance index.

concentration was significantly elevated when rats switched from a HF diet to a LF, LF+BM or HF+BM diet (*P* < 0.05, Table 5).

DISCUSSION

The present study demonstrated that BM reduced weight gain and body fat without affecting energy intake and apparent fat absorption in rats fed a HF diet. BM lowered serum insulin and leptin but raised serum FFA concentration. The impaired oral glucose tolerance that accompanied fat feeding was normalized by BM supplementation. The data suggest that BM altered energy balance through its effects on fat metabolism. Improved insulin resistance might be secondary to a reduced visceral fat mass. These multiple effects of BM are novel findings, and the results support the view that BM is a useful dietary adjunct for the management of body weight and glucose intolerance.

Bitter melon (BM) is a widely cultivated plant for food and medicinal uses. The medicinal properties of its fruit, seeds, vines and leaves are recorded in Ben Cao Gang Mu, an ancient Chinese pharmacopoeia (3). In folklore medicine, BM juice is used as a herbal remedy for a variety of ailments including colic, gout and diabetes (19). Among the documented effects of BM (20,21), its hypoglycemic effect perhaps

has received the most attention. Results of the present study indicate an improvement in insulin resistance in rats fed the HF diet and also provide new evidence for a role of BM in body weight management. Because glycemic control is related in part to weight (fatness) (22), the high fat feeding regimen allowed us to examine these two variables during consumption of BM. In the dose-response study, female rats were used (Experiment 1). The rationale was that if BM affects body weight, the effect might be better demonstrated in slow-growing adult females. Although body weight statistics were hampered by the larger than anticipated variability, a dose-dependent decrease in EE that was significant at the highest dose of BM was noted (Table 2). Because the oral glucose tolerance of rats fed the HF diet was improved when BM was added at a dose ≥0.75% (Fig. 1), the effects of 0.75% BM were tested again in male Sprague-Dawley rats, which in our experience, have more consistent responses to a HF diet. In Experiment 2, BM clearly reduced adiposity (Table 3) and improved insulin resistance (Table 5) of the rats fed the HF diet.

The deceleration in body weight gain in BM-supplemented rats was associated with lower EE. Because neither energy intake nor absorption efficiency was altered, less efficient metabolic processes are implicated in the HF+BM rats. In Experiment 2, a LF or a LF+BM diet replaced the habitual HF diet

TABLE 5

Effects of a low fat (LF) diet with or without bitter melon (BM) on hormone and substrate concentrations in male rats previously exposed to a high fat (HF) diet (Experiment 2)^{1,2}

	LF	HF/LF	HF/LF+BM	HF	HF/HF+BM
<i>n</i>	7	8	8	8	8
Visceral fat pad weight, g	45.4 ± 4.6 ^a	49.9 ± 3.9 ^a	46.9 ± 3.3 ^a	79.3 ± 4.5 ^c	66.9 ± 4.3 ^b
Liver weight, g	20.3 ± 0.8 ^a	22.1 ± 0.9 ^{ab}	21.3 ± 0.8 ^a	24.1 ± 1.0 ^b	20.9 ± 0.8 ^a
Final plasma glucose, mmol/L	6.9 ± 0.2 ^a	6.8 ± 0.1 ^a	6.5 ± 0.1 ^a	7.7 ± 0.2 ^b	6.5 ± 0.2 ^a
Serum FFA, mmol/L	0.73 ± 0.03 ^{ab}	0.93 ± 0.08 ^c	0.92 ± 0.05 ^c	0.63 ± 0.06 ^a	0.82 ± 0.07 ^{bc}
Serum insulin, pmol/L	361.6 ± 58.6 ^a	371.8 ± 55.4 ^a	305.9 ± 58.2 ^a	570.8 ± 60.4 ^b	372.4 ± 58.7 ^a
IRI, 10 ⁻³ pmol insulin × mmol glucose/L ²	2.55 ± 0.44 ^a	2.57 ± 0.39 ^a	2.02 ± 0.38 ^a	4.40 ± 0.51 ^b	2.45 ± 0.42 ^a
Serum leptin, µg/L	1.91 ± 0.37 ^a	2.30 ± 0.28 ^a	2.10 ± 0.31 ^a	6.76 ± 0.64 ^c	5.10 ± 0.74 ^b
Serum TNF-α, ng/L	21.8 ± 3.2	25.8 ± 6.9	25.9 ± 8.3	16.5 ± 2.7	16.3 ± 4.0

¹ Refer to Table 3 footnotes for details.

² Results are expressed as means ± SEM. Means in each row not sharing a letter are significantly different, *P* < 0.05. FFA, free fatty acids; IRI, insulin resistance index; TNF-α, tumor necrosis factor-α.

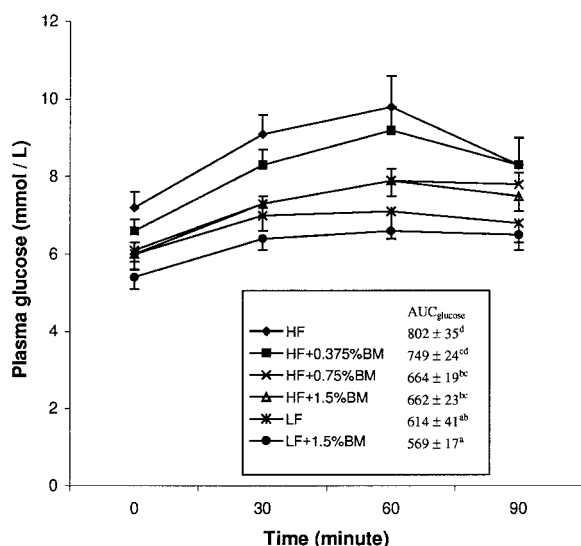


FIGURE 1 Effects of freeze-dried bitter melon (BM) juice supplementation on oral glucose tolerance in rats fed low (LF) and high fat (HF) diets (Experiment 1). Female rats were given LF or HF diets for 6 wk. BM supplementation began at wk 7 and the oral glucose tolerance test was performed at wk 12. Rats were given glucose water (20 g/100 g, 7.5 mL/kg) 8 h after the removal of food cups. Results are expressed as means \pm SEM. There was group effect [$F(5,37) = 8.189, P < 0.001$] on area under the curve for glucose (AUC_{glucose}; mmol \times min/L). Means without a common letter differ, $P < 0.05$.

in two groups of rats. This design was used to further examine the effect of BM on rats during deceleration in weight gain. The result was somewhat unexpected. Weight gain (and fat deposition) of the HF/LF+BM group was not different from that of the HF/LF group (Tables 3 and 5). Therefore, BM appeared to have lost its effect in rats that were already engaged in a metabolic process leading to a lower EE. Whether a higher dose would make a difference awaits testing.

The mechanisms whereby BM lowers EE and reduces visceral fat mass accumulation remain unknown. Changes in substrate and hormone concentrations, however, suggest that the low insulin environment that accompanied BM supplementation might favor lipolysis. Indeed, serum FFA concentration was increased in rats fed the BM-supplemented HF diet (Table 5). Others have reported lower triglyceride levels in plasma (14) and liver (13,14) in BM-treated rats. Taken together, these data suggest that BM may suppress lipogenesis (LF group) or lipid deposition (HF group) but favor fat mobilization. The FFA might become substrates for the uncoupling protein-mediated adaptive thermogenesis that is important to the maintenance of energy balance in rodents (23,24). In Experiment 2, serum FFA concentration increased by 30% and EE decreased by 14% when the HF/HF+BM group was compared with the HF group. The magnitude of the change was similar to the 27% increase and 16% decrease in serum FFA and EE, respectively, when the HF/LF group was compared with the LF group. We contend that a high FFA concentration might be an integral part of a physiologic process that lowers metabolic efficiency.

With a few exceptions (12,25,26), the hypoglycemic effect of BM has been well documented. The controversial results are likely due to variations in the method of preparation (fruit with or without seeds; aqueous or organic extract) and dosing scheme (gavage or addition to the diet). In Experiment 1, the doses selected [equivalent to 5, 10 and 20 mL fruit juice/(kg

body weight \cdot d)] were based on the reported improvement in glucose tolerance when animals were given fruit juice (10 mL per kg) orally once a day for 5 d (27) or 10 wk (11,14). In a separate study (data not shown), however, we did not observe a hypoglycemic effect at wk 3 of BM (0.75%) supplementation. The data suggest, therefore, that different mechanisms of action might mediate the acute and chronic effects of BM on glycemia.

Over the years, several mechanisms have been put forward to explain the hypoglycemic effect of BM. For instance, a fraction that competitively inhibits intestinal glucose uptake has been identified (9). Others have shown that BM extracts stimulated insulin release from isolated pancreatic islet cells (28,29). The improvement in glucose tolerance, however, is independent of an increase in blood insulin (7,30–33). The presence of insulinomimetic agent(s) in BM is supported by the isolation of an 11-kDa protein (p-insulin) which is hypoglycemic when given subcutaneously to human diabetics (10). To the best of our knowledge, the present study is the first to demonstrate that BM improves insulin resistance (lower IRI) and reverses high fat diet-induced glucose intolerance (reduced AUC_{glucose}). The present data, however, suggest that an insulinomimetic action, if one exists, would be minor because serum FFA concentration was increased rather than decreased. We hypothesize that the hypoglycemic effect of BM is secondary to the metabolic environment associated with reduced adiposity.

The potential involvements of leptin and TNF- α were also explored. Both hormones appear to play a role in insulin resistance (34). A positive correlation was observed between serum insulin and leptin (Experiment 1, $r = 0.72, df = 41, P < 0.01$; Experiment 2, $r = 0.51, df = 37, P < 0.01$). Because the relationship disappeared when visceral fat mass was controlled, the metabolic effects are likely not initiated by leptin. Although TNF- α could influence leptin production (35), no

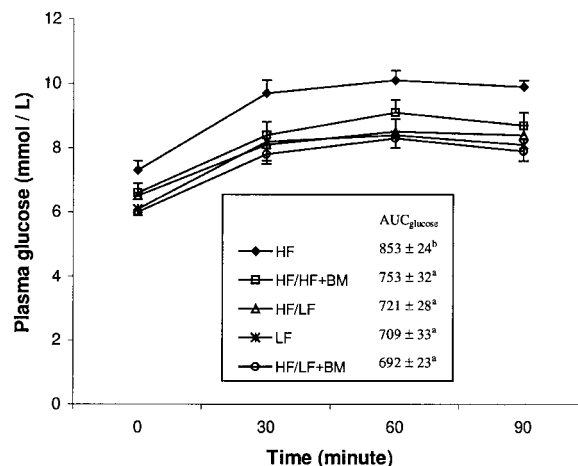


FIGURE 2 Effects of switching from a high fat (HF) diet to HF+ freeze-dried bitter melon (BM) juice, low fat (LF) or LF+BM diet on oral glucose tolerance in male rats (Experiment 2). Rats were given either a low fat diet ($n = 7$) or a high fat diet ($n = 32$) for 4 wk. At the beginning of wk 5, the HF rats either continued to consume the HF diet or were randomly assigned to one of the following diets: low fat (HF/LF), low fat with 0.75% BM (HF/LF+BM), high fat with 0.75% BM (HF/HF+BM). At wk 10, rats that had been food deprived for 8 h were given glucose water (20 g/100 g, 7.5 mL/kg). Results are expressed as means \pm SEM. There was group effect [$F(4,34) = 5.311, P < 0.01$] on area under the curve for glucose (AUC_{glucose}; mmol \times min/L). Means without a common letter differ, $P < 0.05$.

correlation between the two hormones was observed (Experiment 2: $r = -0.16$, $P = 0.325$). Nevertheless, a positive correlation between serum TNF- α and serum FFA ($r = 0.34$, $df = 37$, $P < 0.01$) supports a lipolytic role of TNF- α (36).

In summary, BM alters energy balance through its effects on fat metabolism. The improved insulin resistance could be secondary to a decrease in visceral fat content. The observed effects are likely induced by more than one bioactive compound present in BM. Thus, dietary BM supplementation offers an alternate approach for studying the complex relationships among energy balance, adiposity and endocrine functions.

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