Supplementation of Bitter Melon to Rats Fed a High-Fructose Diet During Gestation and Lactation Ameliorates Fructose-Induced Dyslipidemia and Hepatic Oxidative Stress in Male Offspring

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Abstract

This study examined the impact of maternal high-fructose intake and if metabolic control in the offspring could benefit from supplementing bioactive food components such as bitter melon (BM) to the maternal diet. In Expt. 1, virgin female rats received control (C), high-fructose (F; 60%), or BM-supplemented fructose (FBM; 1%) diet before conception until d 21 of lactation. Weaned male offspring were fed the C diet for 11 wk, forming C/C, F/C, and FBM/C groups. The F/C group had elevated serum insulin, TG, and FFA concentrations and hepatic lipid alterations compared with the C/C and FBM/C groups (P, 0.05). The 2 latter groups did not differ. Expt. 2 had similar dam treatment groups, but offspring were weaned to the C or F diet, forming C/C, C/F, F/F, and FBM/F groups, and the dietary treatment was extended to 20 wk. The hepatic levels of stearoyl-CoA desaturase and microsomal TG transfer protein mRNA were lower, but that of PPARγ coactivator 1-α and fibroblast growth factor 21 mRNA and fatty acid binding protein 1 protein were higher in the FBM/F group compared with the C/F and F/F groups (P, 0.05), indicating that maternal BM supplementation may reduce lipogenesis and promote lipid oxidation in offspring. The FBM/F group had significantly higher activities of liver glutathione peroxidase, superoxide dismutase, and catalase than the F/F group. The results indicate that supplementing BM to dams could offset the adverse effects of maternal high-fructose intake on lipid metabolism and antioxidant status in adult offspring. J. Nutr. 141: 1664–1672, 2011.

Introduction

The concept of “developmental origins of adult disease,” often called “developmental programming,” hypothesized (1) that environmental factors including maternal nutrition experienced in utero and during early postnatal life can elicit permanent metabolic and physiological modifications in individuals, leading to enhanced susceptibility to develop diseases later in life (2,3). In earlier studies that explored the impact of maternal nutrition, manipulation of energy, protein, or fat received much attention (2–5). We are interested in the effects of fructose, because over the past 40 y, its consumption has climbed dramatically and coincided with the prevalence of obesity and diabetes worldwide (6,7). Numerous studies indicated that fructose overconsumption could have deleterious metabolic effects in humans (8,9) and animals (10,11), leading to the development of components of metabolic syndrome, including insulin resistance, dyslipidemia, and hypertension. Thus, the effects of maternal F4 intake on the risk of metabolic derangement in the
offspring, which is still poorly characterized, may be of great importance.

A logical question that arises is whether adverse consequences in offspring exposed to maternal dietary and/or metabolic insults could be offset through the provision of BFC to mothers. The literature suggests that this is feasible, because perinatal supplementation of micronutrients (12) or l-arginine plus antioxidants, including vitamin C, vitamin E, and taurine (13), were shown to ameliorate hypertension in adult offspring. On the other hand, excessive multivitamin intake during pregnancy could accelerate the development of obesity in offspring fed an obesogenic diet (14). Many BFC have been shown to possess antimetabolic syndrome activity. For instance, berry fruits such as strawberry (14). Many BFC have been shown to possess antimetabolic syndrome activity. For instance, berry fruits such as strawberry (14). Many BFC have been shown to possess antimetabolic syndrome activity. For instance, berry fruits such as strawberry (14). Many BFC have been shown to possess antimetabolic syndrome activity. For instance, berry fruits such as strawberry (14). Many BFC have been shown to possess antimetabolic syndrome activity. For instance, berry fruits such as strawberry (14). Many BFC have been shown to possess antimetabolic syndrome activity. For instance, berry fruits such as strawberry (14). Many BFC have been shown to possess antimetabolic syndrome activity. For instance, berry fruits such as strawberry (14). Many BFC have been shown to possess antimetabolic syndrome activity. For instance, berry fruits such as strawberry (14).

Hence, the objectives of the present study were to examine the impact of a fructose-rich maternal diet and test the hypothesis that metabolic control of the offspring benefits from the addition of BFC such as BM to the maternal diet. To our best knowledge, the effect of BM on developmental programming has not been examined. We first established the model of a maternal dietary fructose-induced metabolic profile in male offspring that consumed the C diet postweaning and demonstrated the ability of maternal BM supplementation to offset the metabolic perturbations. In Expt. 2, we explored the concept of 2-generation F exposure. All offspring were fed the F diet to simulate the easy accessibility to fructose-containing food such as soft drinks and fruit juices in modern societies. Serum and hepatic lipids, hepatic expression of genes and proteins related to lipid metabolism, and activities of antioxidant enzymes were measured.

Materials and Methods

Preparation of freeze-dried BM juice powder

BM juice powder was prepared as previously described (20). BM juice was frozen and completely dried after 72 h of freeze-drying operation (Dura Bulk Tray Dryer, FTS System). The yield of juice powder was ~16 g/kg of fresh fruit and the BM powder was stored at ~70°C.

Experimental diets

Diet (Supplemental Table 1) were based on the AIN-93G formulation (26). The C and F diets contained 63% (wt:wt) cornstarch and 60% (wt: wt) fructose, respectively. The FBM diet contained 1% (wt:wt) BM powder and this dose is the median of an effective BM dosage range (0.75–1.25%) we established earlier (18,20). Fructose and BM were added at the expense of cornstarch component. All diets contained 5% lard (27,28) and 2% corn oil to avoid essential fatty acid deficiency. The energy density of the experimental diets was ~16.4 kJ/g. Fresh diets were prepared regularly and stored at 4°C.

Animals

The animal protocol was approved by the Committee on the Use of Live Animals in Teaching and Research at the University of Hong Kong. Two replicate experiments were performed to generate pregnant females. In each experiment, virgin female Sprague-Dawley rats (3 wk old, 45–65 g) were obtained from the Laboratory Animal Unit. They were individually housed under a 12-h-light/-dark cycle at 22 ± 2°C with water and the C diet available ad libitum. One week after arrival, rats were randomly assigned to the C, F, or FBM diet (n = 8/group in Expt. 1 and n = 9/group in Expt. 2). Eight weeks later, they were randomized to C, F, or FBM diets (12 wk old, 450–500 g). On the day that a copulation plug was detected, the pregnant female was isolated. They continued to receive their respective diets throughout gestation and lactation. Food intake and BW were monitored periodically.

The date of birth was referred to as postnatal d 0. In Expt. 1, the total number of live births from the C-, F-, and FBM-fed dams (8 dams/group) was 123, 110, and 110, respectively. The corresponding number of live births in Expt. 2 was 117, 114, and 114 (9 dams/group), respectively. The litter size and birth weight were recorded and the number of pups (gender not yet identified) was standardized to 8/litter by excluding the very heavy and light pups within 24 h after birth to minimize the variation in nutritional intake during suckling. During lactation BW of dams and offspring was monitored twice a week. At the end of lactation (d 21), dams were feed deprived for 12 h and killed by decapitation. At weaning (postnatal d 21), one male pup (with median BW) was selected from each litter and given the C or F diet. Thus pups within each offspring group were born to different dams and hence minimized the “litter effects,” as recommended for developmental nutritional studies (29). Female offspring were used for a separate experiment and thus only data on male offspring are reported here.

Expt. 1.

Male offspring weaned to the C diet. Three groups were studied, namely C/C, F/C, and FBM/C (n = 7/group), with the letters denoting the diet of the dam and offspring, respectively. Offspring were individually housed with food intake and BW monitored twice per week for 11 wk. At the end of study (i.e., 14 wk of age), rats were killed by decapitation after 12-h overnight feed deprivation.

Expt. 2.

Male offspring weaned to the F diet. The design intends to simulate the scenario in affluent societies in which fructose overconsumption occurs in 2 consecutive generations (i.e. 2-generation F exposure). Offspring of C-fed dams were fed the C or F diet and that of F- and FBM-fed dams were fed the F diet, forming the C/C, C/F, F/F, and FBM/F groups (n = 9). To allow the adverse effects of F diet on offspring to manifest, the duration of the feeding period was extended to 20 wk.

For dams and adult offspring, blood and tissues were collected for substrate determination and biochemical analysis.

Measurements in serum and liver

The concentrations of serum glucose (glucose oxidase kit, Sigma), TG (E-test kit, Stanbio), CHL (E-test kit, Thermo), and FFA (NEFA C-test kit, Wako) were measured using commercial assay kits. The serum insulin concentration was measured using an ELISA kit according to the manufacturers’ instructions (Merckodia).

Hepatic lipid was extracted according to Folch et al. (30). Briefly, lipid extract was obtained using chloroform/methanol (2:1, v:v) and redissolved in tert-butanol/Triton X-100. The hepatic TG (E-test kit, Stanbio) and CHL (E-test kit, Thermo) concentrations were determined using commercial kits.

RNA isolation and RT-PCR

Genes involved in lipogenesis, including sterol regulatory element binding transcription factor 1 (SREBP1), fatty acid synthase (FAS), acetyl-Coenzyme A carboxylase beta (ACAC2), stearoyl-CoA desaturase (SCD), and microsomal triglyceride transfer protein (MTP), as well as genes involved in fatty acid oxidation, including PPARα, PPAR-gamma coactivator 1-alpha (PGC1α), and fibroblast growth factor 21 (FGF21), were measured as target genes. Total RNA was extracted from liver by TRizol reagent (Invitrogen) according to the protocol provided by the
manufacturer. First-strand cDNA was synthesized by using oligo(dT) primer (Invitrogen) with Moloney Murine Leukemia Virus RT (Invitrogen). cDNA was amplified using β-Taq DNA polymerase (Bio-Firm) and sequence-specific sense and antisense primers (Invitrogen). The primer pairs used in PCR are shown in Supplemental Table 2. The band intensities were quantified by image analysis (ImageJ software). Each value was normalized to that for the housekeeping gene, β-actin.

Western-blot analysis
Western blotting was performed as previously described (31). Briefly, liver (0.1 g) was homogenized with lysis buffer. Total tissue lysate was obtained by differential centrifugation. Protein concentrations of liver samples were determined by the method of Bradford (32). Liver lysates (15 µg) were resolved by 12.5% SDS-PAGE gel and subjected to immunoblotting using anti-VCALD (Abcam), anti-α-fatty acid binding protein 1 (FABP1) (Abcam), anti-CPT1α (Santa Cruz), and anti-α-tubulin (Santa Cruz) antibodies. α-Tubulin was used as internal loading control to verify the loading of protein was equal. Protein bands on the membrane were visualized by Enhanced Chemiluminescence Reagent (Amersham). The band intensities were quantified by ImageJ software.

Analysis for hepatic antioxidant enzymes
GST activity was measured by the method previously described (33), using 1-choro-2,4-dinitrobenzene (Sigma) as substrate. One unit of GST was defined as the amount of the enzyme catalyzing the conjugation of 1 µmol 1-choro-2,4-dinitrobenzene/min. GR activity was assayed by measuring the oxidation rate of NADPH (34). One unit of GR was defined as the amount of the enzyme oxidizing 1 nmol of NADPH/min. GPx activity was determined by the coupled assay described previously (35) in which tert-butyl hydroperoxide was used as substrate. One unit of GPx was defined as 1 nmol of NADPH oxidized/min. SOD activity was assayed by the inhibition of the formation of adrenochrome from autooxidation of epinephrine (36). One unit of SOD activity was defined as percent inhibition of epinephrine oxidation. Catalase activity was measured by the method of Aebi (37). The activity was calculated based on the decomposition rate of hydrogen peroxide. The protein concentration of liver extracts was determined by the method of Bradford (32) and all enzyme activities were expressed as units/mg protein.

Statistical analysis
Results are reported as means ± SEM. Data of dams and offspring were separately analyzed by 1-way ANOVA followed by post hoc Dunnet’s test (SPSS for Windows, version 12.0) to determine the treatment effect and compare mean difference among the groups. Bivariate correlations between antioxidant enzyme activities and serum FFA concentration were calculated using the Pearson correlation coefficient. Difference was considered significant at P < 0.05.

Results

Effects of supplementing BM to dam’s F diet

Expt. 1. BW and food intake. Virgin female rats were fed the C, F, or FBM diet for 8 wk before mating and during pregnancy and lactation. Weight gain of female rats did not differ among the groups before conception (202 ± 3 g), during 19 d of pregnancy (113 ± 4 g), or during 21 d of lactation (~5 ± 3 g). Total energy intake of female rats did not differ among the groups before conception (17.0 ± 0.2 MJ) or during 19 d of pregnancy (7.0 ± 0.1 MJ).

Serum and liver lipids analysis. At the end of lactation, the F-fed dams had higher serum TG (0.73 ± 0.04 mmol/L) and CHL (2.88 ± 0.24 mmol/L) concentrations compared with the C-fed dams (TG: 0.50 ± 0.04 mmol/L; CHL: 1.74 ± 0.16 mmol/L) (P < 0.05). The FBM-fed dams had a lower serum CHL concentration (2.10 ± 0.20 mmol/L) (P < 0.05) but a similar serum TG concentration (0.73 ± 0.07 mmol/L) compared with the F-fed dams. Dietary treatments did not affect the serum glucose and insulin concentrations among the groups (data not shown).

The liver weight did not differ among the C-fed dams (9.2 ± 0.3 g) and FBM-fed dams (11.4 ± 0.4 g) (P < 0.05). The hepatic TG and CHL concentrations in the F-fed dams (TG: 22.6 ± 0.1 µmol/g; CHL: 18.1 ± 1.3 µmol/g) was 30 and 171% higher, respectively, than that of the C-fed dams (TG: 17.4 ± 1.1 µmol/g; CHL: 6.7 ± 0.4 µmol/g) (P < 0.05), whereas these concentrations in the FBM-fed dams (TG: 19.2 ± 0.9 µmol/g; CHL: 8.9 ± 0.7 µmol/g) were less than that in the F-fed dams (P < 0.05).

Expt. 2. Because virgin female rats were given the same dietary treatments, the results were comparable, with 2 exceptions, to that of Expt. 1 (Supplemental Table 3). In this experiment, the F-fed dams gained more weight (P < 0.05) compared with both the C- and FBM-fed dams before conception. The serum TG concentration also did not differ among the groups at the end of lactation.

Male offspring weaned to the C diet: Expt. 1

BW, energy intake, and biochemical data. The litter size (14.3 ± 0.4) and birth weight (6.5 ± 0.1 g) did not differ among the dam groups. Beginning on postnatal d 21, male offspring of dams fed the C, F, or FBM diet were fed the C diet for 11 wk. There were no differences in the BW, total energy intake, and serum glucose and CHL concentrations among the groups (Table 1). The serum insulin, TG, and FFA concentrations in the F/C group were higher than the C/C group by 67, 34, and 30%, respectively (P < 0.05; Table 1). Maternal BM supplementation lowered the serum insulin, TG, and FFA concentrations in the F/C group (P < 0.05; Table 1).

Liver weight and lipids. The liver weight did not differ among the groups (Table 1). The F/C group had higher hepatic TG and

| TABLE 1 | Body weight (BW), food intake, and serum and liver profiles in male offspring of control (C)-fed dams that consumed the C diet postweaning (C/C), of high-fructose (F)-fed dams that consumed the C diet (F/C), and of bitter melon-supplemented F diet-fed dams that consumed the C diet (FBM/C) offspring at 14 wk of age (Expt. 1) |
|----------|-------------------------------------------------|---------------------------------|-----------------|-----------------|-----------------|
| Initial BW (d 21), g | 63 ± 2 | 61 ± 2 | 58 ± 3 |
| BW at wk 11, g | 557 ± 13 | 543 ± 30 | 542 ± 24 |
| Weight gain, g/11 wk | 494 ± 14 | 482 ± 29 | 483 ± 22 |
| Energy intake, mL/11 wk | 36.9 ± 1.1 | 36.9 ± 2.1 | 35.4 ± 1.6 |
| Serum insulin, µmol/L | 1.27 ± 0.16| 212 ± 30* | 141 ± 23* |
| Serum glucose, mmol/L | 8.7 ± 0.3 | 9.2 ± 0.3 | 8.6 ± 0.3 |
| Serum TG, mmol/L | 1.40 ± 0.07* | 1.67 ± 0.09* | 1.13 ± 0.12* |
| Serum CHL2, µmol/L | 2.69 ± 0.18 | 3.09 ± 0.23 | 2.84 ± 0.10 |
| Serum FFA, µmol/g | 0.50 ± 0.04* | 0.65 ± 0.03* | 0.47 ± 0.04* |
| Liver weight, g | 15.1 ± 0.6 | 16.2 ± 1.2 | 14.8 ± 0.8 |
| Liver TG, µmol/g tissue | 17.6 ± 1.0* | 20.6 ± 0.6* | 17.9 ± 0.7* |
| Liver CHL, µmol/g tissue | 8.2 ± 0.2* | 9.2 ± 0.3* | 8.0 ± 0.2* |

1 Values are means ± SEM, n = 7. Means in a row with superscripts without a common letter differ, P < 0.05. 2 CHL, cholesterol.
CHL concentrations compared with both the C/C and FBM/C groups (P < 0.05; Table 1).

mRNA and protein expression of markers related to lipid metabolism in the liver. For lipogenic genes, the F/C group had greater hepatic ACC2 mRNA expression compared with the C/C group, whereas the FBM/C group had lower SREBP1 as well as ACC2 expression compared with the F/C group (Fig. 1A, B; P < 0.05). The FAS mRNA expression did not differ among the groups (Fig. 1C).

The hepatic expression of genes involved in fatty acid transport and oxidation was determined (data not shown) and followed by Western-blot analysis. The FABP1 protein expression did not differ among the groups (Fig. 2A). The VLCAD protein expression in the F/C group was lower than both the C/C and FBM/C groups (P < 0.05; Fig. 2B). The FBM/C group also had higher CPT1a protein expression compared with all other groups (P < 0.05; Fig. 2F).

Male offspring weaned to the F diet: Expt. 2

BW, energy intake, and biochemical data. There were no differences in the litter size (14.4 ± 0.3) and birth weight (6.5 ± 0.1 g) among the dam groups. The duration of the postweaning dietary treatments was extended to 20 wk.

The BW, total energy intake, as well as serum glucose and TG concentrations did not differ among the groups (Table 2). Compared with the C/C group, only the C/F group had a higher serum CHL concentration (P < 0.05; Table 2). The serum FFA concentration of the F/F group was higher than that of the C/F group (P < 0.05; Table 2), which in turn was higher than that of the C/C and FBM/F groups (P < 0.05; Table 2). The F/F group, but not C/F, had a higher serum insulin concentration compared with the C/C group (P < 0.05; Table 2). However, the serum insulin concentration of the FBM/F group remained elevated and did not differ from the F/F group (Table 2).

Liver weight and lipids. The liver weight did not differ among the groups (Table 2). Compared with the C/C group, only the C/F group had a greater hepatic TG concentration (P < 0.05; Table 2). Interestingly, the hepatic CHL concentration was lower in the F/F group compared with all other groups (P < 0.05; Table 2).
mRNA expression of markers related to lipogenesis in the liver. The hepatic expression of SREBP1, ACC2, and FAS mRNA was greater in the C/F group compared with the C/C group by 45, 167, and 44%, respectively (P < 0.05; Fig. 3A–C). The expression of these 3 genes in both the F/F and FBM/F groups did not differ compared with the C/C group. For genes regulating lipoprotein synthesis and assembly (38,39), the SCD and MTTP mRNA expression was greater in the C/F and F/F groups compared with both the C/C and FBM/F groups (P < 0.05; Fig. 3D,E).

mRNA and protein expression of markers related to fatty acid oxidation in the liver. The mRNA expression of PPARα and PGC1α, a central regulator and transcriptional coactivator for fatty acid oxidation (40,41), respectively, in both the C/F and F/F groups was lower than that in the C/C group (P < 0.05; Fig. 4A,B). However, the FBM/F group had greater PGC1α mRNA expression compared with both the C/F and F/F groups and also had greater mRNA expression of FGFR21, a PPAR-dependent gene implicated as a novel metabolic regulator (42), compared with all other groups (P < 0.05; Fig. 4B,C).

The FABP1 protein expression was lower in both the C/F and F/F groups compared with the C/C group (P < 0.05; Fig. 5B). However, the FBM/F group had greater FABP1 protein expression compared with both the C/F and F/F groups (P < 0.05; Fig. 5B). Compared with the C/C group, only the C/F group had lower VLCAD protein expression (P < 0.05; Fig. 5D).

Activities of antioxidant enzymes in the liver. The prooxidant effects of excessive fructose intake and the impact of BM were evaluated by measuring the activities of a battery of antioxidant enzymes in the liver of the offspring.

The C/F group had lower GST, GPx, and SOD activities compared with the C/C group (P < 0.05; Table 3). The F/F group also had lower GST, GPx, and catalase activities compared with the C/C group (P < 0.05; Table 3). Although the GST activity of the FBM/F group was lower than that of the C/C group, activities of the other 4 antioxidant enzymes were similar between the 2 groups (Table 3). The FBM/F group had higher activities of GPx, SOD, and catalase compared with the F/F group (P < 0.05; Table 3). The FBM/F group also had higher activity of GR, GPx, and SOD compared to the C/F group (P < 0.05; Table 3).

Among all the offspring (n = 36), the activities of GST (r = −0.42; P = 0.01), GPx (r = −0.43; P = 0.009), and catalase (r = −0.44; P = 0.008) were negatively correlated with serum FFA concentration.

### Discussion

In this study, we investigated the impacts of maternal fructose excess and tested our hypothesis that metabolic control in the

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**TABLE 2** Body weight (BW), food intake, and serum and liver profiles in male offspring of control (C)-fed dams that consumed the C diet postweaning (C/C), of C-fed dams that consumed the high-fructose (F) diet (C/F), of F-fed dams that consumed the F diet (F/F), and of bitter melon-supplemented F diet-fed dams that consumed the C diet (FBM/F) at 23 wk of age (Expt. 2)1

<table>
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<tr>
<th></th>
<th>C/C</th>
<th>C/F</th>
<th>F/F</th>
<th>FBM/F</th>
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<tr>
<td>Initial BW (d 21), g</td>
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<td>62 ± 2</td>
<td>64 ± 1</td>
<td>64 ± 1</td>
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<td>BW at wk 20, g</td>
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<td>Weight gain, g/20 wk</td>
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<td>571 ± 13</td>
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<td>596 ± 17</td>
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<td>Energy intake, MJ/20 wk</td>
<td>72.9 ± 1.4</td>
<td>74.0 ± 2.3</td>
<td>68.6 ± 2.7</td>
<td>72.2 ± 1.4</td>
</tr>
<tr>
<td>Serum insulin, pmol/L</td>
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<td>1.16 ± 12b</td>
<td>237 ± 25b</td>
<td>229 ± 21b</td>
</tr>
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<td>Liver TG, g/20 wk</td>
<td>7.2 ± 0.2</td>
<td>7.9 ± 0.2</td>
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<tr>
<td>Serum glucose, mmol/L</td>
<td>0.68 ± 0.03</td>
<td>0.76 ± 0.02</td>
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<td>Liver weight, g</td>
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<td>Liver CHL, µmol/g tissue</td>
<td>27.3 ± 3.5</td>
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<td>22.1 ± 2.4</td>
<td>33.0 ± 5.7</td>
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<td>Liver FFA, µmol/g tissue</td>
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<td>9.7 ± 0.8</td>
<td>6.5 ± 0.4</td>
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1 Values are means ± SEM, n = 9. Means without a common superscript differ, P < 0.05.

**FIGURE 3** Liver SREBP1 (A), ACC2 (B), FAS (C), SCD (D), and MTTP (E) mRNA expression in the male offspring of control (C)-fed dams that consumed the C diet postweaning (C/C), of C-fed dams that consumed the high-fructose (F) diet (C/F), of F-fed dams that consumed the F diet (F/F), and of bitter melon-supplemented F diet-fed dams that consumed the F diet (FBM/F) at 23 wk of age (Expt. 2). Quantitative data are band densities relative to that of β-actin. Values are means ± SEM, n = 9. Means without a common letter differ, P < 0.05.

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offspring would benefit from the addition of BM to the maternal diet. The key findings are: 1) maternal F exposure resulted in dyslipidemia and hepatic lipid accumulation, whereas BM provided protection against these fructose-induced metabolic abnormalities in the dams and their offspring that consumed the C diet postweaning; 2) when offspring were weaned to the F diet, those born to F-fed dams (i.e., 2-generation F exposure) had elevated serum insulin and FFA concentrations, altered lipid metabolism, and compromised antioxidant status in the liver; 3) except hyperinsulinemia, maternal BM supplementation ameliorated the metabolic changes associated with 2-generation F exposure.

Maternal malnutrition has been implicated as a contributing factor to the prevalence of obesity and metabolic syndromes (2–5), but improving the intrauterine environment through dietary interventions can benefit the offspring later in life (43,44). In this study, the phenotypic changes in F/C offspring are consistent with the evidence that maternal fructose diet predisposed the offspring to an array of metabolic disorders (45,46). To our knowledge, however, this is the first study reporting that maternal BM supplementation effectively ameliorated the fructose-induced adverse metabolic effects in both dams and adult offspring. We demonstrated that BM supplementation lowered cholesterolemia in F-fed dams and also lowered triglyceridemia and insulinemia in their F/C offspring. These BM-mediated effects are consistent with our earlier observations (18,19) and that of others (22) when animals that received obesogenic diets were supplemented with BM.

Aggravated metabolic dysfunctions in the offspring have been reported when rodents were fed a high-fat diet or junk food for 2 generations (47,48). Our feeding paradigm did further provoke insulin resistance with the findings that the serum insulin and FFA concentrations were higher in F/F offspring than in C/C and C/F offspring. Surprisingly, the hepatic TG concentration was not affected and the CHL concentration was even lower in F/F offspring than in C/C offspring. It is possible that the flux of FFA may be diverted to other insulin-sensitive tissues such as skeletal muscle instead of liver. The lower hepatic CHL is unlikely explained by reduction of hepatic CHL uptake and synthesis, because their serum CHL concentration and hepatic expression of SREBP2 and 3-hydroxy-3-methylglutaryl-CoA reductase mRNA were not affected (data not shown). Because the hepatic CHL level is also regulated by the rate of biliary CHL secretion (49), this may be a contributing factor that warrants further investigation.

Although lowering serum FFA can improve insulin sensitivity (50), we found disparity in this association when maternal BM supplementation was applied to the 2-generation F exposure model. That is, despite the serum FFA concentration of FBM/F offspring was restored to the level observed in C/C offspring, hyperinsulinemia remained in the former group (Table 3). We speculate that supplementing BM to F-fed dams may benefit their offspring via either repressing formation of enhancing oxidation of FFA and also improve insulin sensitivity via modifying insulin action rather than insulin secretion (22) in F/F offspring. Collectively, our data indicate that BM could fully reverse the fructose-induced phenotypic changes imposed on the offspring.
only if they were weaned to the C diet. The differential outcomes of maternal BM supplementation highlight the importance of the postweaning diet. Whether 2-generation F exposure results in an irreversible damage to glycemic control remains to be elucidated.

Fructose stimulates hepatic lipogenesis and inhibits fatty acid oxidation (9). Hence, when deducing BM’s mechanism of action, we focused on these 2 aspects. Lower ACC2 mRNA expression and higher CPT1α and VCLAD protein expression observed in FBM/C offspring suggest that benefits of maternal BM supplementation could stem from the suppression in lipogenesis and enhancement in lipid oxidation. This notion is supported by the results of Expt. 2 in which FBM/F offspring had suppressed expression of SCD and MTTP but enhanced expression of PPAR-regulated genes and proteins, including FG Fel and FABP1 and the coactivator PGC1α. The data are consistent with the responses on PPAR-responsive murine hepatoma cells treated with an ethyl acetate BM extract (51).

Interestingly, the mRNA expression of lipogenic genes, including SREBP1, FAS, and ACC2 in F/F offspring, was not affected by maternal BM supplementation. Both F/F and FBM/F offspring had lower expression of these genes compared with C/F offspring. These suppressive responses are in keeping with the concept of predictive adaptive response whereby a developing baby responds to maternal suboptimal conditions, including malnutrition, through modifications in the prediction of future environment in attempt to maximize survival postnatally (2,52). Furthermore, although higher SCD mRNA expression and lower PPARα and PGC1α mRNA as well as FABP1 protein expression were observed in F/F offspring, these molecular changes did not match their phenotypic profile in liver lipids. That is, F/F offspring do have the capacity to increase lipogenesis and reduce lipid oxidation, but the liver lipids content remained suppressed. To address these discrepancies, future works in protein phosphorylations and enzyme activities are needed. Another focus could be hepatic VLDL production, because F/F offspring had higher mRNA expression of MTTP, which may lead to the increase of assembly and secretion of apoB-lipoproteins (10,53).

Dietary fructose creates a prooxidant environment and the induced oxidative stress has been linked to an array of metabolic and endocrine abnormalities (11,54). Both C/F and F/F offspring had reduced activities of liver antioxidant enzymes, which are consistent with the observations that catalase activity is lower in rats fed a fructose-rich diet for 3 wk (55). These abnormalities, however, are reversible, because the activities of GR, GPx, SOD, and catalase in FBM/F offspring were restored to the levels observed in C/C offspring. Similar observations were reported in diabetic animals after BM supplementation (23,24). We speculate that lipid peroxidation may play a role, because the serum malondialdehyde concentration in FBM-fed rats was similar to C-fed rats and significantly lower than F-fed rats (Supplemental Table 3). Furthermore, the negative relationships between serum FFA, a known inducer of oxidative stress (56,57), and liver antioxidant enzyme activities (GST, GPx, and catalase) reinforce the notion that hepatic antioxidant capacity is linked to lipid metabolism.

Although innovative in design, this study did have some limitations. First, because BM was given throughout gestation and lactation, the critical time of benefit could not be pinpointed. Second, the BM preparation is a crude extract and the bioactive component(s) have not been isolated. It is not known if such component(s) acted on the dams only or could be passed onto the fetus and neonate through placenta or milk. Third, maternal protein restriction altered methylation status in hepatic glucocorticoid receptor and PPARα genes leading to persistent phenotypic changes in rat offspring. Notably, these changes were normalized by maternal folic supplementation (58). Thus, the possibility that the BM-mediated effects observed in the offspring may be mediated by epigenetic regulation warrants future investigation.

In conclusion, our present findings support the concept of developmental programming, because metabolic abnormalities observed in the F-fed dams were replicated as hyperinsulinemia, deranged lipid metabolism, and compromised antioxidant status in the offspring. That these abnormalities were largely reversed by supplementing BM to F-fed dams highlights the significance of perinatal dietary intervention that favorably modifies metabolic risks later in life.

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