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Maternal green tea extract supplementation to rats fed a high-fat diet ameliorates insulin resistance in adult male offspring☆

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Abstract

Maternal overnutrition is associated with increased risk of metabolic disorders in the offspring. This study tested the hypothesis that maternal green tea (GT) supplementation can alleviate metabolic derangements in high-fat-diet-fed rats born of obese dams. Female Sprague–Dawley rats were fed low-fat (LF, 7%), high-fat (HF, 30%) or HF diet containing 0.75% or 1.0% GT extract (GT1, GT2) prior to conception and throughout gestation and lactation. Both doses of GT significantly improved metabolic parameters of HF-fed lactating dams ($P<.05$). Birth weight and litter size of offspring from HF dams were similar, but GT supplementation led to lighter pups on day 21 ($P<.05$). The weaned male pups received HF, GT1 or GT2 diet (dam/pup diet groups: LF/HF, HF/HF, HF/GT1, HF/GT2, GT1/HF and GT2/HF). At week 13, they had similar weight but insulin resistance index (IRI), serum nonesterified fatty acid (NEFA) and liver triglyceride of rats born to GT dams were 57%, 23% and 26% lower, accompanied by improved gene/protein expressions related to lipid and glucose metabolism, compared with the HF/HF rats ($P<.05$). Although HF/GT1 and HF/GT2 rats had lower serum NEFA, their insulin and IRI were comparable to HF/HF rats. This study shows that metabolic derangements induced by an overnourished mother could be offset by supplementing GT to the maternal diet and that this approach is more effective than giving GT to offspring since weaning. Hence, adverse effects of developmental programming are reversible, at least in part, by supplementing bioactive food component(s) to the mother's diet.

Keywords: Developmental programming; High-fat; Green tea; Insulin resistance; Offspring

1. Introduction

A growing body of literature suggests a causal link between maternal and child obesity [1–3]. With the increasing proportion of overweight and obesity in women of child-bearing age [4], it is pertinent to investigate the role of maternal overnutrition and explore potential intervening strategies. To this end, emerging evidence [5–8] suggests that the increased risk of chronic disease in later life is related, at least in part, to the process of developmental programming originally proposed by Hales and Baker to explain the impact of undernutrition *in utero* and early postnatal life [9].

Numerous studies have demonstrated a potential role for bioactive food components (BFCs) as an adjunct to the treatment of obesity and metabolic syndrome [10,11]. Common to these BFCs is that most possess strong antioxidant properties. Indeed, a recent study had demonstrated a protective role of supplementing antioxidants to the dam's diet. In the latter study, maternal-obesity-induced oxidative imbalance was restored when Western-diet-fed dams received a mixture of antioxidant supplement, and that benefit in offspring was a decrease in adiposity [12]. The type of vitamin supplement obviously is critical because others have reported increase in adiposity in young rats fed an obesogenic diet when dams received excessive amount of vitamin during pregnancy [13].

Our interest in tea stems from the fact that it is a popular beverage for many cultures and is consumed worldwide. The health benefits of tea have appeared in numerous reviews, and the effects are usually attributable to the catechins [14,15]. Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea. Being an insulin potentiating factor and antioxidant, EGCG is postulated to play a role in preventing metabolic syndrome [15]. Dietary EGCG supplementation reduced body fat in male mice [16], and EGCG injected into lean and obese Zucker rats significantly reduced blood glucose and insulin levels [17]. Dietary EGCG supplementation also reduced body weight as well as plasma glucose, insulin and lipids in human [18,19].

To our best knowledge, the effect of GT on developmental programming has not been studied. Hence, this study aimed to test the hypothesis that the beneficial effects of green tea extract (GTE) on metabolic control of dams would pass on to the offspring as adults, that is, the metabolic derangements in offspring born to high-fat-diet-fed dams could be ameliorated if dams are supplemented with GTE. Different from most programming studies in which the offspring were normally fed a control diet, in the present study, the male pups were also given a high-fat diet. This two-generation fat exposure design aims to simulate the worse case scenario in our modern societies. We

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first established the impacts of maternal high fat in the presence and absence of GTE supplementation on dams and then compared the effects of prenatal vs. postweaning GTE supplementation on male offspring. Males were selected because the programming effects on insulin response appear to be gender specific, with insulin resistance and hyperinsulinemia readily observed in male pups born of obese dams [20]. In this study, two doses of GTE were added to the maternal HF diet or postweaning HF diet separately, generating six maternal/postweaning diet combinations: LF/HF, HF/HF, HF/GT1, HF/GT2, GT1/HF and GT2/HF pups. The glucose, insulin concentration and lipid profile of adult male were monitored, and changes in their metabolic regulation by maternal dietary manipulations were described in the context of relevant mRNA/protein expressions in liver, adipose tissues and muscle.

2. Materials and methods

2.1. GTE and experimental diets

The GTE was a commercial product (Teavigo) purchased from DSM Nutrition Products Ltd., Switzerland. It contains a minimum of 90% EGCG.

The formulation of diets was based on the AIN-93G recommendation [21]. The control diet was the low-fat (LF) diet which contained corn oil (70 g/kg), casein (200 g/kg), corn starch (529.5 g/kg), mineral mix (35 g/kg) and vitamin mix (10 g/kg). The high-fat (HF) diet contained corn oil (70 g/kg), Crisco shortening (230 g/kg; Procter & Gamble, Cincinnati, OH, USA), casein (235 g/kg), corn starch (255.5 g/kg), mineral mix (42 g/kg) and vitamin mix (12 g/kg). In addition, all diets contained sucrose (100 g/kg), cellulose (50 g/kg) and miscellaneous ingredients (2.5 g/kg choline bitartrate, 3.0 g/kg L-cystine and 0.014 g/kg *tert*-butylhydroquinone). Green tea extract was added to the HF diet at the expense of cornstarch. GT1 and GT2 diets contained, respectively, 7.5 g and 10 g GTE/kg. Energy density of the LF and HF diet was 16.5 and 21.1 MJ/kg, respectively.

2.2. Animal experiments

All animal protocols were approved by the Committee on the Use of Live Animals in Teaching and Research at The University of Hong Kong (no. 1949-09). Virgin female Sprague–Dawley rats, at 4 weeks of age, and male Sprague–Dawley rats (for mating), at 7 weeks of age, were obtained from the animal unit of the Medicine Faculty. Rats were housed individually and kept in a controlled environment at 22°C under a 12-h-light/12-h-dark cycle with light on from 7:00 a.m. to 7:00 p.m. Water and laboratory food (Lab Diet, The Richmond Standard; PMI Nutritional International Inc., St. Louis, MO, USA) were available *ad libitum* to male rats at all time and female rats for a few days before they were assigned to their respective diet groups. Food intake and body weight were measured two to three times weekly.

The female rats (n=8 per group) were given LF, HF, GT1 or GT2 diet for 8 weeks. Tail blood was collected at the end of week 8. Starting at week 9, female rats and male rats (450–500 g) were housed together. On the day that a copulation plug was found (referred to as G1 in Table 1), the pregnant female was separately housed and remained on its original diet throughout gestation and lactation. At parturition, the pups were weighed, and litter size was standardized to eight per dam. At weaning [postpartum day (PPD) 21], male pups (with median body weight [BW]) were selected from each litter and weaned to the HF, GT1 or GT2 diet. Hence, there were six maternal/postweaning diet groups: LF/HF, HF/HF, HF/GT1, HF/GT2, GT1/HF and GT2/HF. These rats were kept on their respective diet for 10 weeks. Both the dams and offspring were killed by decapitation in the absence of anesthesia at the end of lactation (PPD 21) and week 13 of age, respectively. Blood was collected from the cervical wound into chilled test tube and centrifuged (2000*g* at 4°C). Serum collected together with liver, visceral fat [22], brown adipose tissue and the red medial part of gastrocnemius [23] were stored at −80°C.

Table 1
Body weight, energy intake, energy efficiency and fat mass of dams [1]

	LF	HF	GT1	GT2
Before mating				
Body weight (week 1), g	116±6	120±3	119±3	118±3
Body weight (week 8), g	311±10^{b}	345±10^{a}	295±8^{bc}	278±5^{c}
Weight gain (week 1–8), g	195±13^{b}	225±8^{a}	176±8^{bc}	160±4^{c}
Energy intake (week 1–8), MJ	16.3±0.3^{ab}	17.2±0.3^{a}	15.6±0.4^{bc}	15.0±0.5^{c}
Energy efficiency (week 1–8), g gain/MJ consumed	11.9±0.6^{ab}	13.1±0.3^{a}	11.3±0.4^{bc}	10.6±0.3^{c}
Gestation				
Body weight (G1), g	319±12^{ab}	333±9^{a}	290±8^{b}	289±13^{b}
Body weight (G15),[2] g	417±19	427±16	378±14	379±19
Weight gain (G1–15), g	98±11	94±8	88±8	89±10
Energy intake (G1–21), MJ	8.3±0.7	9.5±0.4	9.4±0.3	8.3±0.3
Lactation				
Body weight (PPD1), g	380±16^{ab}	408±17^{a}	354±14^{b}	346±14^{b}
Weight change (PPD1–21), g	−25±7^{bc}	−46±7^{c}	−12±8^{ab}	4±7^{a}
Energy intake (PPD1–21), MJ	17.1±1.2	14.4±1.3	15.2±0.7	14.4±0.9
Visceral fat (PPD21), g	14.8±1.7^{ab}	18.0±2.5^{a}	11.2±2.1^{b}	11.6±1.6^{b}

[1] Values are means±S.E.M., n=8. Means within a row lacking a common superscript letter^{a,b,c,d} differ (P<.05).

[2] To avoid disturbing the dams near parturition, body weight of dams was only measured up to day 15 of gestation.

2.3. Biochemical assays

Plasma glucose was assayed immediately using a commercially available glucose oxidase kit (kit no. 510-A, Sigma Chemical). Serum insulin was determined by enzyme-linked immunosorbent assay (ELISA) kit (Mercodia rat insulin 10-1124-01; Mercodia AB, Uppsala, Sweden). The insulin resistance index (IRI) was calculated as the product of insulin and glucose concentration (10^{-3} pmol insulin · mmol glucose · L^{-2}) for each individual rat [24].

Serum, liver and muscle triglyceride (TG) were measured using Triglyceride LiquiColor (2200-430, Stanbio Laboratory). Cholesterol was measured using Cholesterol Liquid Stable Reagent (TR13421, Thermo Scientific). Serum nonesterified fatty acid (NEFA) was measured using Labassay NEFA (294-63601, Wako). Serum leptin and adiponectin were determined by ELISA kits (EZRL-83K, EZRADP-62K, Linco, USA).

2.4. RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR) analysis

RNA was extracted by the TRIZOL reagent according to manufacturer's protocol (Invitrogen). First-strand cDNAs were synthesized from 2 μg of total RNA using 200 U of M-MLV reverse transcriptase (28025-021, Invitrogen), RNaseOUT Recombinant Ribonuclease Inhibitor (10777-019, Invitrogen), oligo (dT) primers (18418-012, Invitrogen) and dNTP mix (US77212, Amersham Biosciences). The PCR was performed in the presence of Taq DNA polymerase with PCR buffer and $MgCl_2$ (Bio-firm), dNTP mix (US77212, Amersham Biosciences), and forward and reverse primers (Invitrogen). Sequences of the primers used in the RT-PCR reaction are shown in Table S1.

2.5. Western blotting analysis

The liver and gastrocnemius muscle tissues were homogenized with a tissue teaser (Biospec Products) in a lysis buffer (1% Triton X-100, 25 mmol/L HEPES, 150 mmol/L NaCl, 1 mmol/L EDTA disodium salt, 1 mmol/L dithiothreitol) with added protease inhibitors: 2 μg/ml aprotinin, 10 μg/ml leupeptin and 1 mM phenylmethylsulfonyl fluoride (Usb). Samples were centrifuged at 600*g* for 10 min to pellet the nuclei. Supernatants were centrifuged at 16,000*g* for 20 min. The cytosolic fraction was collected. Protein concentration in the samples was measured using the Bradford Reagent (Bio-Rad). Fifty micrograms of protein of each sample was applied for electrophoresis on 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels and transferred to polyvinylidene diflouride membrane (GE Healthcare). Membranes were blocked with 5% nonfat dried milk powder in TBST buffer (50 mmol/L Tris-HCl, pH 7.4, 0.15 mol/L NaCl, .05% Tween 20) for 2 h. The membranes were then incubated for 2 h with anti-PGC1α (ab72230, Abcam) (1:1000), anti-GLUT4 (sc-1608, Santa Cruz) (1:1000), anti-SREBP1 (sc-13551, Santa Cruz) (1:1000), anti-G6pase (sc-33839, Santa Cruz) (1:1000) and anti-tubulin (sc-5286, Santa Cruz) (1:4000). α-Tubulin was used as an internal control to verify equal loading of the protein. Horseradish-peroxidase-conjugated secondary antibodies (sc-2004, sc-2031, sc-2020, Santa Cruz) were used. Membranes were developed using ECL Western blotting reagents (Amersham Biosciences), and the chemiluminescence was detected on Hyperfilm ECL (Amersham Biosciences). The band intensities were measured with Quantity One software (Bio-Rad).

2.6. Statistical analysis

Data are expressed as means±S.E.M. Analyses were carried out using SPSS 12.0 for Windows. Data were analyzed by analysis of variance followed by post hoc Duncan's multiple range tests to determine treatment effect and compare differences among group means. Differences were considered significant at P<.05.

3. Results

3.1. Weight gain, body fat and energy efficiency

Before mating, GTE-supplemented females gained less weight and had lower energy efficiency than HF females (Table 1, P<.05). The GT1 rats had similar weight gain and energy efficiency compared to the LF rats, while the GT2 rats gained less and had lower energy efficiency than the LF rats (Table 1, P<.05). During gestation, weight gain and

Table 2
Serum, liver and muscle parameters of dams at the end of lactation (PPD21) [1]

Parameter	LF	HF	GT1	GT2
TG, mg·dl^{-1}	58.5±1.4^{b}	72.3 ±5.4^{a}	47.6±4.1^{b}	50.2±1.8^{b}
Cholesterol, mg·dl^{-1}	61.0±1.1^{b}	71.4±4.7^{a}	56.0±2.7^{b}	56.6±4.2^{b}
NEFA, mEq·L^{-1}	1.01±0.07^{ab}	1.09±0.10^{a}	0.76±0.09^{b}	0.79±0.07^{b}
Insulin, μg·L^{-1}	0.13±0.01^{b}	0.18±0.03^{a}	0.11±0.01^{b}	0.10±0.01^{b}
Glucose, mg·dl^{-1}	167±8	166±8	166±5	162±7
IRI, 10^{-3} pmol·mmol·L^{-3}	0.22±0.02^{ab}	0.29±0.04^{a}	0.18±0.02^{b}	0.16±0.01^{b}
Leptin, ng·ml^{-1}	1.3±0.2^{b}	2.4±0.3^{a}	1.0±0.1^{b}	1.2±0.2^{b}
Adiponectin, ng·ml^{-1}	18.4±3.0	14.2±3.0	18.3±4.0	17.6±1.4
Liver TG, mg/g tissue	12.4±0.4^{b}	16.0±1.1^{a}	10.5±0.8^{b}	10.8±1.5^{b}
G TG, mg/g tissue	2.3±0.3^{b}	6.1±0.7^{a}	2.8±0.4^{b}	3.4±0.3^{b}

[1] Values are means±S.E.M., n=8. Means within a row lacking a common superscript letter^{a,b,c,d} differ (P<.05).

energy intake were not different between the GTE-supplemented and HF dams (Table 1). One day postpartum (PPD1), GTE-supplemented dams were lighter than the HF dams (Table 1, P<.05), but not different from the LF dams. All dams lost weight throughout the lactation period, but loss was significantly less in the GTE-supplemented dams than the HF dams (Table 1, P<.05). Energy intake among the four groups, however, was not different. At the end of lactation (PPD21), the GTE dams had less visceral fat than the HF dams (Table 1, P<.05).

On PPD1, the body weight and litter size of offspring born to the HF dams and the GTE dams were not different (Table 3). But on PPD21, offspring from the GTE dams weighed less than those from the HF dams (Table 3, P<.05). At week 13, there was no difference in body weight and visceral fat mass among the six groups. The GT2/HF group, however, demonstrated higher energy efficiency than the HF/HF group (Table 3).

3.2. Glucose and insulin sensitivity

On PPD21, the HF dams had higher serum insulin concentration than the LF dams (Table 2). GTE supplementation decreased serum insulin in the HF dams to the level of the LF dams. There was no difference in plasma glucose concentration among the LF, HF and GTE dams (Table 2).

At week 13, male rats born to the LF dams (LF/HF) tended to have lower serum insulin concentration and IRI compared with those from HF dams (HF/HF, HF/GT1 and HF/GT2). And rats born to the GTE-supplemented dams (GT1/HF and GT2/HF) had lower serum insulin concentration and IRI than those born to the HF dams (HF/HF) (P<.05), while rats from the HF dams who received GTE supplementation postweaning did not experience reduction in serum insulin and IRI (HF/GT1 and HF/GT2 vs. HF/HF) (Table 3). Concentration of serum glucose among the GT1/HF, HF/HF, HF/GT1 and LF/HF rats was not different, but the GT2/HF and HF/GT2 rats had lower serum glucose concentration compared to the HF/HF rats (Table 3, P<.05).

3.3. Lipid profiles in serum, liver and muscle and serum adipocytokines

At the end of lactation (PPD21), the HF dams had elevated serum TG and cholesterol, as well as liver and gastrocnemius muscle TG, compared with the LF dams. But both doses of GTE supplementation ameliorated these abnormalities (Table 2). GTE supplementation also lowered serum NEFA in the HF dams. Furthermore, the HF dams had higher serum leptin concentration than the LF dams, while GTE supplementation lowered the leptin concentration in the HF dams (Table 2, P<.05).

At week 13, male rats born to the HF dams (HF/HF) had higher serum, liver and gastrocnemius muscle TG compared with those from the LF dams (LF/HF), while maternal GTE supplementation ameliorated these abnormalities in the HF offspring (GT1/HF and GT2/HF). Maternal GTE supplementation also lowered serum cholesterol and NEFA in the HF offspring (GT1/HF and GT2/HF vs. HF/HF) (Table 3, P<.05). Postweaning GTE supplementation alone (HF/GT1 and HF/GT2) resulted in lower serum TG, NEFA and cholesterol compared with the HF/HF rats. However, there was no difference in liver and gastrocnemius muscle TG concentration among the HF/GT1, HF/GT2 and HF/HF rats (except that the HF/GT2 rats had lower muscle TG than the HF/HF rats) (Table 3).

3.4. mRNA expression

The HF dams had elevated mRNA expression of hepatic glucose-6-phosphatase (G6pase) and sterol regulatory element binding protein (SREBP-1c), but decreased mRNA expression of adiponectin in white adipose tissue (WAT) and insulin receptor (IR) in liver as well as IR and PGC-1α in gastrocnemius (G), compared with the control dams (Fig. 1, P<.05). Both GTE supplements normalized mRNA expression of hepatic G6pase and SREBP-1c, as well as muscle PGC-1α and IR. The

Table 3
Effects of maternal and postweaning diets on growth and biochemical parameters of male offspring at week 13 [1]

Lactation [2]	LF		HF		GT1	GT2
Litter size (PPD1) [3]	12±1		14±1		13±1	13±1
BW (PPD1), g	7.0±0.1^{a}		6.5±0.1^{b}		6.4±0.1^{b}	6.5±0.1^{b}
BW (PPD21), g	63.8±0.9^{a}		62.3±0.9^{a}		55.0±1.2^{b}	56.0±0.8^{b}
Postweaning	LF/HF	HF/HF	HF/GT1	HF/GT2	GT1/HF	GT2/HF
BW (week 13), g	523±23	558±19	516±21	520±19	552±17	576±15
EI [4], MJ	30.0±1.3^{ab}	30.0±0.9^{ab}	27.1±1.1^{c}	27.3±1.8^{bc}	30.8±0.7^{a}	29.8±0.7^{abc}
EE [5], g gain/MJ consumed	15.6±0.4^{c}	16.4±0.3^{bc}	16.9±0.2^{ab}	16.7±0.3^{ab}	16.0±0.2^{bc}	17.2±0.2^{a}
Insulin, μg·L^{-1}	1.0±0.2^{ab}	1.2±0.2^{a}	1.4±0.2^{a}	1.3±0.2^{a}	0.6±0.1^{b}	0.6±0.1^{b}
Glucose, mg·dl^{-1}	180±10^{a}	169±11^{a}	158±9^{ab}	137±13^{b}	160±7.0^{ab}	135±5^{b}
IRI, 10^{-3} pmol·mmol·L^{-3}	1.77±0.44^{ab}	2.01±0.41^{a}	2.20±0.31^{a}	1.72±0.35^{ab}	0.92±0.16^{b}	0.82±0.17^{b}
Adiponectin, ng/ml	22.6±1.9^{a}	14.7±1.5^{b}	19.9±1.3^{a}	21.7±0.8^{a}	20.0±1.5^{a}	18.9±0.7^{a}
NEFA, mEq·L^{-1}	0.73±0.03^{ab}	0.81±0.05^{a}	0.61±0.05^{b}	0.63±0.05^{b}	0.60±0.05^{b}	0.64±0.05^{b}
TG, mg·dl^{-1}	96.0±4.1^{b}	122.9±5.8^{a}	88.8±8.9^{b}	78.7±9.7^{b}	84.0±7.0^{b}	97.6±10.8^{b}
Cholesterol, mg·dl^{-1}	99.0±4.4^{ab}	108.3±4.7^{a}	88.2±3.0^{b}	86.5±3.1^{b}	93.9±4.3^{b}	92.7±5.6^{b}
Liver TG, mg/g tissue	21.1±0.5^{bc}	23.0±0.6^{a}	22.0±0.4^{ab}	21.6±0.4^{ab}	20.2±0.4^{c}	18.6±0.5^{d}
G TG, mg/g tissue	4.3±0.2^{d}	7.3±0.5^{a}	6.4±0.3^{ab}	4.1±0.4^{d}	5.4±0.5^{bc}	5.2±0.3^{cd}
Visceral fat, g	32±3	41±3	29±3	31±3	37±3	36±3

[1] Values are means±S.E.M., n=7–8. Means within a row lacking a common superscript letter^{a,b,c,d} differ (P<.05).
[2] Dam's diet during lactation.
[3] Litter size per dam on PPD1.
[4] EI=energy intake, from week 4 to week 13.
[5] EE=energy efficiency, from week 4 to week 13.

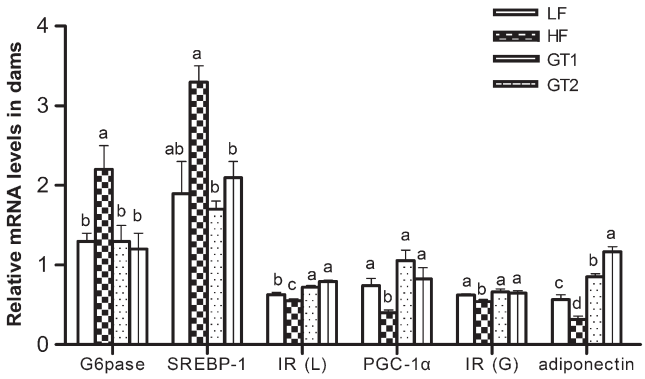

Fig. 1. Relative mRNA levels of liver (L) G6pase, SREBP-1 and IR, gastrocnemius (G) PGC-1α and IR, and WAT adiponectin in LF-, HF-, GT1- and GT2-diet-fed dams on PPD21. Each bar represents mean±S.E.M, n=8. Means without a common letter differ, P<.05.

maternal supplementations also increased mRNA expression of hepatic IR and WAT adiponectin (Fig. 1, P<.05).

Compared with the HF/HF rats, rats born to the GTE-supplemented dams had mRNA expression reduced for hepatic G6pase and SREBP-1c. Maternal GTE supplements, however, restored or further increased mRNA expression for GLUT4 and adiponectin in WAT, uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) and hepatic IR (P<.05) to that of the LF/HF rats. Maternal 0.75% GTE supplementation also restored muscle mRNA expression for PGC-1α, IR and GLUT4 to that of the LF/HF offspring.

GTE supplementation in the postweaning diet led to lower hepatic SREBP-1c in male offspring born to the HF dams (HF/GT1 and HF/GT2 vs. HF/HF; P<.05). One percent GTE in the postweaning diet increased expression of hepatic IR and WAT adiponectin, whereas 0.75% GTE increased IR and PGC-1α in muscle (Fig. 2).

3.5. Protein expression

GTE supplementation to the maternal diet or the postweaning diet lowered the protein level of hepatic G6pase and SREBP-1 in the HF rats (Fig. 3A). And maternal 0.75% GTE supplementation increased the protein level of PGC-1α and GLUT4 in muscle of the HF rats (GT1/HF vs. HF/HF), while 1% GTE supplementation increased protein level of PGC-1α, but not GLUT4 (GT2/HF vs. HF/HF) (Fig. 3B). When added to the postweaning diet, 0.75% GTE increased the protein level of PGC-1α and GLUT4 in gastrocnemius (HF/GT1 vs. HF/HF) (Fig. 3B).

4. Discussion

The powerful effect of developmental programming is clearly manifested in this study. Although all pups were weaned to the HF diet, serum TG and tissue lipid accumulation in offspring born to obese dams were further elevated when compared to those born to nonobese dams. Hence, maternal HF diet could predispose offspring to metabolic syndrome. Our objective was to intervene programming using BFC, and in this context, male offspring born to GTE-supplemented dams exhibited significant improvements in all aspects of metabolic profiles to a condition that is comparable to offspring born to dams fed an LF diet.

Through developmental programming, maternal HF intake can predispose the offspring to glucose intolerance and increase adiposity in adulthood [5,25]. This concept is further substantiated by the present results which employed a unique two-generation fat exposure design to simulate the dietary practice of some population subgroups. Although the HF-diet-fed offspring born to HF-diet-fed dams (HF/HF) were not significantly heavier compared with those from LF-diet-fed dams (LF/HF) at week 13, the former did exhibit significantly higher serum and muscle TG concentration, lower serum adiponectin concentration and 28% more visceral fat mass (Table 3). These results are in keeping with those from our laboratory [26] as well as those others [27,28] in that not all phenotypic features in the offspring derived from the two-generation exposure model would worsen compared with those from one-generation exposure. However, the similar metabolic profiles observed between dams and offspring lend support to the notion of programming effects of

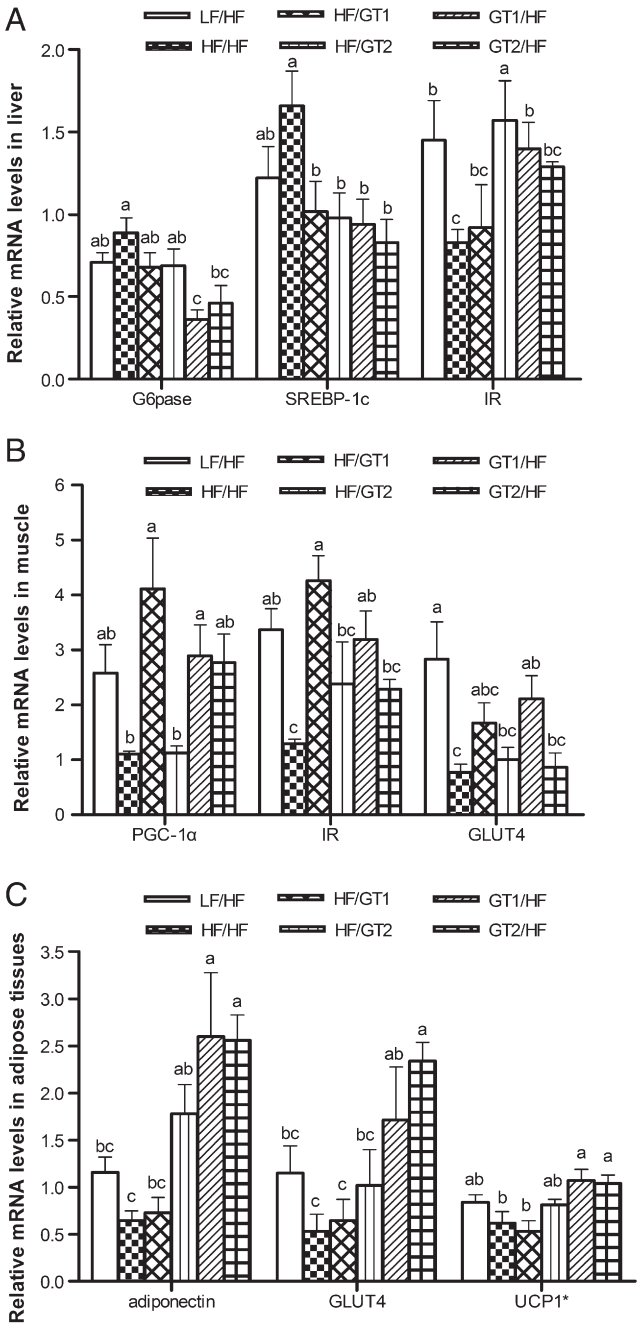

Fig. 2. Relative mRNA levels of genes in (A) liver, (B) gastrocnemius and (C) WAT and BAT* of week 13 male offspring fed with HF, GT1 or GT2 diet (LF/HF, HF/HF, HF/GT1, HF/GT2, GT1/HF and GT2/HF). Each bar represents mean±S.E.M, n=7–8. Means without a common letter differ, P<.05.

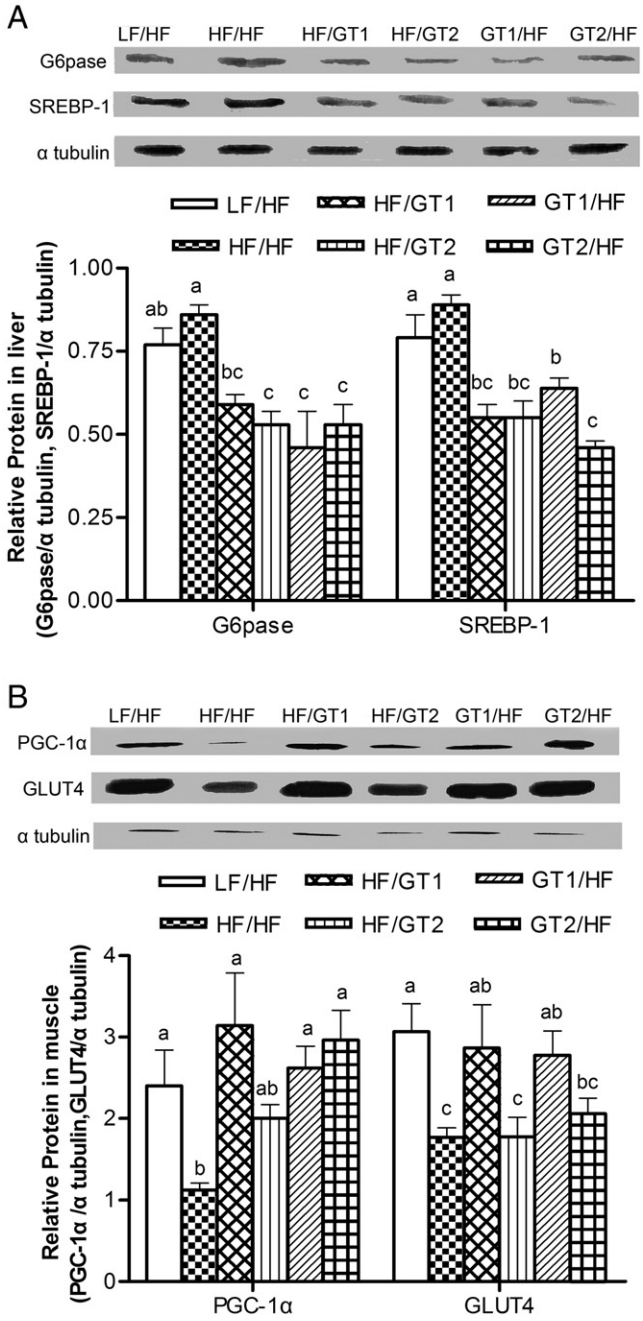

Fig. 3. Protein contents of (A) G6pase and SREBP-1 in liver, and (B) PGC-1α and GLUT4 in gastrocnemius of week 13 male offspring fed with HF, GT1 or GT2 diet (LF/HF, HF/HF, HF/GT1, HF/GT2, GT1/HF and GT2/HF). The protein content was determined by Western blotting. Values are expressed as means±S.E.M, $n=6$. Means without a common letter differ, $P<.05$.

maternal HF diet. Hence, HF-diet-fed dams (HF vs. LF dams, Fig. 1) exhibited a significant decrease in the mRNA expression of IR in liver and muscle, and the same abnormalities were observed in their male offspring (HF/HF vs. LF/HF, Fig. 2A, 2B). The decrease in mRNA and protein abundance of GLUT4 in muscle of the HF/HF rats is consistent with that reported by others [29].

Two doses (0.75% and 1%) of GTE were tested in the present study. The supplemented females exhibited the anticipated [30,31] decrease in body weight and visceral fat mass as well as blood lipids (Tables 1 and 2). Interestingly, decreases in serum lipid, insulin concentration and IRI were also recorded in the 13-week-old male offspring (GT1/HF, GT2/HF vs. HF/HF). These improvements are not related to body weight. Body weight of pups at birth was not affected by maternal GT supplementation. Although pups born to GT dams were lighter than those born to LF or HF dams at weaning, body weight at week 13 was not different among offspring, and in fact, GT2/HF rats caught up fast as they had higher energy efficiency than pups from nonsupplemented dams (GT2/HF vs. HF/HF). Hence, maternal GTE supplementation can mitigate the metabolic derangements in the offspring induced by two-generation fat exposure.

Maternal HF diet and GTE supplementation may alter the fetal environment such as hormonal milieu and epigenetic regulation of gene expression, leading to changes in expression of candidate genes that govern key metabolic processes [32,33]. The present study is the first to report that maternal GTE supplementation induces parallel changes in expression of genes in both dams and their offspring (Figs. 2A, 3A). Hence, hepatic G6pase mRNA expressions were down-regulated, whereas muscle GLUT4 and PGC-1α expressions were elevated. The latter is a transcriptional coactivator [34], and its level has been shown to correlate positively with GLUT4 [35]. Collectively, the data suggest that hepatic glucose production and muscle glucose utilization were, respectively, suppressed and enhanced in the offspring and are consistent with the known effects of catechins on insulin-sensitive tissues [36–38].

Genes related to lipid metabolism and energy homeostasis are also influenced by maternal GTE supplementation. As mentioned earlier, offspring born to HF-diet-fed dams had elevated levels of serum and tissue TG compared to those born to dams fed the LF diet. Such abnormalities were reversed by maternal GTE supplementation, and such phenotypic improvements were accompanied by decreased mRNA level and protein abundance of SREBP-1c in liver of offspring (Figs. 2A and 3A). Our data are therefore in line with the observation that GTE decreased the mRNA expression of SREBP-1c and improved the lipid profile of fructose-fed ovariectomized rats [30]. Adiponectin is implicated in fat metabolism and energy homeostasis. WAT adiponectin mRNA expression was suppressed in HF/HF rats, but such abnormality was reversed by maternal GTE supplementation (Fig. 2C). Increased mRNA expression of adiponectin in adipose tissues was also reported in GTE-supplemented obese dogs [39]. Adiponectin could increase UCP expression [40]. This is consistent with the present results showing an increase in UCP1 expression in BAT of offspring born to GTE-supplemented dams (Fig. 2C).

It is of interest to note that mixed results were obtained when GTE was added to the postweaning diet instead. Although the HF/GT1 and HF/GT2 rats had improved serum lipid profile compared with the HF/HF rats (Table 3), there was no difference in serum insulin and IRI among HF/GT1, HF/GT2 and HF/HF rats (Table 3). These results contrasted those derived from maternal GTE supplementation. Improved serum lipid profile by postnatal GTE supplementation may be explained by decreased expression of hepatic SREBP-1c. On the other hand, postnatal GTE supplementation failed to increase GLUT4 mRNA expression in muscle as well as in adipose tissue or reduce hepatic G6pase expression. In addition, UCP1 expression in BAT was unchanged by postnatal GTE supplementation compared with a significant increase by maternal GTE supplementation (Fig. 2C). These results are in disparity with the well-known effects of GT in the literature and are likely explained by the HF diet fed to pups since weaning in the present study. It is also possible that, in these offspring, improvement in insulin sensitivity might be a latent response that appears later in time. Nevertheless, the current experimental data evidently differentiated the impacts of pre- and postnatal GTE exposure and highlighted the significant role of maternal diet.

It is acknowledged that interpretation of the present data is limited by the nature of the experimental design. First, without a dam group fed GTE-supplemented LF diet, the effects of GTE per se cannot be

determined and will remain associated with the suppressed weight gain during gestation. However, if LF-diet-fed dams were supplemented with GTE at the current levels, they will most certainly reduce energy intake and fat mass, further complicating overall data interpretation. For the purpose of the study, we were able to simulate a metabolic and gene expression profile associate with LF diet in the GTE-supplemented HF-diet-fed dams. Perhaps, in future experiments, lower levels of GTE (0.3% and 0.5%) could be tested. Such doses may help to establish the minimum effective dose and to determine if the GTE effects are entirely attributable to prevention of maternal weight gain. In this study, EGCG exposure in dams receiving the lower dose (0.75%) approximated the no-observed adverse effect level of 500 mg/kg/d [41]. Because catechins are known to cross placenta [42], we could not rule out the possibility that the effects were, at least in part, a direct influence on the developing fetus. Second, the lighter pups (PPD21) born to GT dams compared with those born to LF or HF dams might be the consequence of GTE exposure via milk [43]. To further examine the perinatal effects of GTE supplementation, a subsequent study was performed that aimed to differentiate the effects of GTE supplementation during gestation and lactation (manuscript in preparation).

In conclusion, metabolic derangements induced by overnourished dams could be offset by supplementing GTE to the maternal diet. The current findings provide preliminary evidence to spur further research to fine-tune the exposure level and mechanism of action on developmental programming for many BFCs known to confer health benefits.

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