

Resveratrol supplementation of high-fat diet-fed pregnant mice promotes brown and beige adipocyte development and prevents obesity in male offspring

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Key points

- Maternal high-fat diet impairs brown adipocyte function and correlates with obesity in offspring.
- Maternal resveratrol administration recovers metabolic activity of offspring brown adipose tissue.
- Maternal resveratrol promotes beige adipocyte development in offspring white adipose tissue.
- Maternal resveratrol intervention protects offspring against high-fat diet-induced obesity.

Abstract Promoting beige/brite adipogenesis and thermogenic activity is considered as a promising therapeutic approach to reduce obesity and metabolic syndrome. Maternal obesity impairs offspring brown adipocyte function and correlates with obesity in offspring. We previously found that dietary resveratrol (RES) induces beige adipocyte formation in adult mice. Here, we evaluated further the effect of resveratrol supplementation of pregnant mice on offspring thermogenesis and energy expenditure. Female C57BL/6 J mice were fed a control diet (CON) or a high-fat diet (HFD) with or without 0.2% (w/w) RES during pregnancy and lactation. Male offspring were weaned onto a HFD and maintained on this diet for 11 weeks. The offspring thermogenesis and related regulatory factors in adipose tissue were evaluated. At weaning, HFD offspring had lower thermogenesis in brown and white adipose tissues compared with CON offspring, which was recovered by maternal RES supplementation, along with the appearance of multilocular brown/beige adipocytes and elevated thermogenic gene expression. Adult offspring of RES-treated mothers showed increased energy expenditure and insulin sensitivity when on an obesogenic diet compared with HFD offspring. The elevated metabolic activity was correlated with enhanced brown adipose function and white adipose tissue browning in HFD+RES compared with HFD offspring. In conclusion, RES supplementation of HFD-fed dams during pregnancy and lactation promoted white adipose browning and thermogenesis in offspring at weaning accompanied by persistent beneficial effects in protecting against HFD-induced obesity and metabolic disorders.

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(Received 15 September 2016; accepted after revision 15 November 2016; first published online 28 November 2016)

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Abbreviations AMPK, AMP-activated protein kinase; BAT, brown adipose tissue; CD137, cluster of differentiation 137; Cidea, cell death-inducing DFFA-like effector A; CON, control diet; Cox7a1, cytochrome *c* oxidase subunit VIIa polypeptide 1; Cyto C, cytochrome *c*; Elovl3, elongation of very long-chain fatty acids protein 3; EpiWAT, epididymal white adipose tissue; FABP4, fatty-acid binding protein 4; GTT, glucose tolerance test; H&E, Haematoxylin and Eosin; HFD, high-fat diet; IHC, immunohistochemical; IngWAT, inguinal white adipose tissue; PGC-1 α , peroxisomal proliferator-activated receptor γ coactivator-1; PPAR γ , peroxisomal proliferator-activated receptor γ ; PRDM16, PR domain-containing 16; RES, resveratrol; Sirt1, sirtuin 1; Tbx1, T-box 1; Tmem26, transmembrane protein 26; UCP1, uncoupling protein 1.

Introduction

A growing body of evidence suggests that maternal nutrition and physiological conditions are important determinants of the health outcome of offspring (Borengasser *et al.* 2013; Nathanielsz *et al.* 2013; Giussani *et al.* 2014; Forhead *et al.* 2015). Maternal dietary intake during pregnancy and lactation affects the development of the fetus and neonate, resulting in developmental programming that alters offspring life-course physiology and metabolism (Boekelheide *et al.* 2012; Poston, 2012). Epidemiological and experimental studies show that maternal obesity or high-fat diet (HFD) consumption is associated with increased susceptibility to hepatic steatosis and inflammation (Bruce *et al.* 2009), hypertension and cardiovascular disorders (Samuelsson *et al.* 2008; Blackmore *et al.* 2014; Taylor *et al.* 2014), impaired hippocampal neurogenesis (Tozuka *et al.* 2009; Dearden & Ozanne, 2015), adrenal and thyroid dysfunction (Franco *et al.* 2012), as well as obesity and insulin resistance (Boyle *et al.* 2016) in offspring at different stages of development.

Obesity, characterized by excess adipose tissue accumulation, is a major risk factor leading to metabolic syndrome and type 2 diabetes. In mammals, white adipose tissue (WAT) stores excess energy in the form of triglycerides (Giralt & Villarroya, 2013). In contrast, two populations of uncoupling protein 1 (UCP1)-positive thermogenic adipocytes, referred to as classical brown adipocytes in brown adipose tissue (BAT; Cannon & Nedergaard, 2004) and brite/beige (**b**rown in **w**hite) adipocytes of WAT (Giralt & Villarroya, 2013), dissipate energy directly as heat. Thus, pharmacological and nutritional strategies for increasing BAT thermogenic activity and browning of WAT promote energy dissipation and combat obesity and type 2 diabetes (Bonet *et al.* 2013).

Together with dietary macro- and/or micronutrients, provision of bioactive food components, such as polyphenols, to mothers can programme offspring growth and metabolic pathways, which can further alter lifelong susceptibility to obesity and its related complications (Santangelo *et al.* 2014; Vega *et al.*

2016). Several reports have indicated the metabolic programming effects of maternal polyphenol intake on offspring. For example, grape skin extract was effective in protecting adult offspring against obesity, hypertension and insulin resistance when administered during lactation in rats (Resende *et al.* 2013). Maternal dietary genistein supplementation throughout pregnancy and lactation prevented obesity in mouse offspring (Dolinoy *et al.* 2006). The maternal administration of quercetin during gestation and lactation decreased offspring body weight, and improved insulin sensitivity and lipid metabolism in adipose tissue (Wu *et al.* 2014). Moreover, although offspring from rat dams fed HFD with low doses of grape seed procyanidin during pregnancy and lactation exhibited an increase in adiposity, these animals possessed healthier adipose tissue than the offspring of dams fed HFD (Del Bas *et al.* 2015).

As a nutraceutical dietary supplement, resveratrol (RES), a natural polyphenolic compound commonly found in grape skins and other fruits, has been shown to protect against HFD-induced obesity in mammals (Kim *et al.* 2011; Jeon *et al.* 2012) and exerts health benefits in obese persons (Timmers *et al.* 2011). In addition, maternal RES intake improves fetal outcomes impaired as a result of to hypoxia (Bourque *et al.* 2012), ethanol exposure (Kumar *et al.* 2011), protein restriction (Vega *et al.* 2016), hypertension (Care *et al.* 2016) and valproic acid-induced neurological disorder (Bambini-Junior *et al.* 2014). However, to our knowledge, the effect of RES supplementation of HFD mothers on brown and beige adipose development and thermogenesis in offspring has not been examined.

Proposed anti-obesity mechanisms of RES include decreased adipogenesis (Rayalam *et al.* 2008) and lipogenesis (Li *et al.* 2016), increased lipolysis (Lasa *et al.* 2012), adipocyte apoptosis (Rayalam *et al.* 2008), increased thermogenesis (Wang *et al.* 2015) and enhanced mitochondrial function (Lagouge *et al.* 2006). One plausible explanation for these beneficial effects is the induction of beige adipogenesis, which promotes lipid oxidation to generate heat (Wang *et al.* 2015). Thus, we hypothesized that maternal resveratrol administration

could facilitate brown and beige adipogenesis during early offspring development, and exert beneficial effects on energy metabolism and adaptive thermogenesis in adult offspring.

Methods

Ethical approval

All animal experimental and care procedures were performed in accordance with the guidelines of the National Institutes of Health and approved by the Institutional Animal Use and Care Committee of Washington State University.

Animals and experimental design

Female C57BL/6 J mice (3 months old) were maintained in controlled conditions ($23 \pm 2^\circ\text{C}$, 12 h–12 h light–dark cycle) with free access to food and water. The day of mating was determined by examination of vaginal smears. This day was designated as day 0 of gestation. Pregnant mice were housed individually and randomly divided into four dietary groups ($n = 10$ per group). Two groups were fed with a control diet (CON; 10% energy from fat, D12450H; Research Diets, New Brunswick, NJ, USA) or an identical diet supplemented with resveratrol (CON+RES). The other two groups were fed with an HFD (45% energy from fat, D12451; Research Diets) or an identical diet supplemented with resveratrol (HFD+RES). Resveratrol (Orchid Pharma, Inc., Princeton, NJ, USA) was mixed with either powdered CON or HFD diet at a concentration of 0.2% (dry feed, w/w), and pellets were then reconstituted. All dams were fed their respective diets from day 0 of gestation until weaning (postnatal day 21). In previous studies, dietary supplementation of 0.1–0.4% RES effectively ameliorated HFD-induced adiposity (Lagouge *et al.* 2006; Kim *et al.* 2011; Wang *et al.* 2015). Thus, we chose an average, 0.2% RES, supplementation for our study, which was equivalent to ~ 200 mg (kg body weight) $^{-1}$ day $^{-1}$. As discussed previously, similar doses demonstrated beneficial effects to the health of mothers and their fetuses (Vega *et al.* 2016). Weight gain and food intake were monitored. Litters were culled to six pups within 24 h after birth to ensure adequate and uniform maternal nutrition to pups. Litters with fewer than six pups were removed from the study. On postnatal day 21, pups were weaned, and dams and one male pup from each litter were killed by carbon dioxide inhalation and cervical dislocation. Blood samples were collected by cardiac puncture and centrifuged at 4°C to collect serum. The interscapular BAT, inguinal WAT (IngWAT) and epididymal WAT (EpiWAT) were rapidly isolated and weighed. Tissues from one side were fixed in 4% paraformaldehyde for sectioning and

staining, and from the other side they were rapidly frozen in liquid nitrogen and stored at -80°C until further analyses. After weaning, the remaining male pups were weaned onto HFD (D12451) to mimic a postweaning obesogenic environment. The offspring body weights and food intake were recorded weekly. After 11 weeks of HFD challenge, mice were killed by CO_2 inhalation and cervical dislocation, and blood was collected by cardiac puncture. The liver, BAT, IngWAT and EpiWAT were harvested and weighed.

Glucose tolerance test (GTT) and blood characteristics

At 1 week before the mice were killed, following an overnight fast, mice were injected I.P. with D-glucose (1 g kg^{-1}). Blood samples were collected from the tail vein at 0, 15, 30, 60 and 90 min after injection, and glucose concentrations were measured using a glucometer (Bayer Contour, Tarrytown, NY, USA), and the area under the curve was quantified (Um *et al.* 2010; Fu *et al.* 2015). Serum insulin concentration was measured using a Mouse Ultrasensitive Insulin ELISA Kit (no. 80-INSMSU-E10; ALPCO Diagnostics, Salem, NH, USA), and triglycerides were determined using the triglyceride colorimetric assay kit from Cayman (no. 10010303; Ann Arbor, MI, USA).

Whole-body metabolic analysis

The whole-body metabolic rate [oxygen consumption (\dot{V}_{O_2}), carbon dioxide production (\dot{V}_{CO_2}), respiratory exchange ratio (RER) and heat production] was measured using an Oxymax indirect open-circuit calorimetry system (Columbus Instruments, Columbus, OH, USA) installed in a constant environmental temperature and light regimen (12 h light and 12 h dark). Mice in each chamber had free access to food and water.

Acute cold tolerance test

Mice were individually housed in precooled cages and exposed to a cold temperature (4°C) for 6 h with free access to food and water. Their rectal temperature was measured hourly using a digital thermometer (Thermalert TH-5; Physitemp, Clifton, NJ, USA).

Histological analysis

Fresh adipose tissues were fixed for 24 h at room temperature in PBS containing 4% paraformaldehyde, and embedded in paraffin. Then, $5\text{-}\mu\text{m}$ -thick tissue sections were deparaffinized, rehydrated and either stained with Hematoxylin and Eosin (H&E) or used for UCP1 immunohistochemical (IHC) staining as previously described (Wang *et al.* 2015). At least four images per

Table 1. Primer sequences used for real-time quantitative PCR

Gene	Forward (5'–3')	Reverse (5'–3')	Size (bp)	Accession no.
<i>18S</i>	GTAACCCGTTGAACCCATT	CCATCCAATCGGTAGTAGCG	151	NR_046233.2
<i>UCP1</i>	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG	123	NM_009463.3
<i>PRDM16</i>	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG	87	NM_001291029.1
<i>Cidea</i>	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGCCATTCT	136	NM_007702.2
<i>Elovl3</i>	GATGGTTCTGGGCACCATCTT	CGTTGTTGTGTGGCCTCTT	73	XM_006526624.1
<i>Cox7a1</i>	CAGCGTCATGGTCACTCTGT	AGAAAACCGTGTGGCAGAGA	112	NM_009944.3
<i>PGC-1α</i>	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCTGTTTTTC	161	XM_006503779.1
<i>CD137</i>	GTCGACCCTGGACGAACTGCTCT	CCTCTGGAGTCACAGAAATGGTGTA	132	NM_001077590.1
<i>Tbx-1</i>	TGAAGAAGAACCCGAAGGTGG	ACTTGAACGTGGGGAACATT	133	XM_006536887.1
<i>TMEM26</i>	GAAACCAGTATTGCAGCACCCAAT	AATATTAGCAGGAGTGTGGTGGA	205	NM_177794.3
<i>PPARγ</i>	AGCTCCAAGAATACCAAAGTGCGAT	AGTTCCTCATGAGGCTGTTGTAGA	98	XM_017321456.1
<i>FABP4</i>	CGACAGGAAGGTGAAGAGCATCATA	CATAAACTCTGTGGAAGTCAAGCCT	158	NM_024406.2

section and four sections from each individual mouse were analysed. Adipocyte diameters were measured by Image-Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA; Liang *et al.* 2016).

Quantitative real-time PCR

Total RNA was extracted from different adipose tissues using TRIzol reagent (Sigma, Saint Louis, MO, USA) according to the manufacturer's instructions, and cDNA was synthesized from 500 ng of total RNA using the iScriptTM cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Real-time quantitative PCR was carried out using the CFX RT-PCR detection system (Bio-Rad) as described previously with *18S* rRNA used as a reference gene (Wang *et al.* 2015). The relative mRNA expression was determined using the method of $2^{-\Delta\Delta C_T}$ (Livak & Schmittgen, 2001). The primer sequences are listed in Table 1.

Immunoblotting analysis

Immunoblotting analysis was conducted as previously described using an Odyssey Infrared Image System (LI-COR Biosciences, Lincoln, NE, USA; Wang *et al.* 2015). Antibodies against AMP-activated protein kinase α (AMPK α ; no. 2532), phospho-AMPK α at Thr172 (no. 2535), cytochrome *c* (Cyto C; no. 4280), Sirtuin 1 (Sirt1; no. 2028), peroxisomal proliferator-activated receptor γ (PPAR γ ; no. 2443) and β -tubulin (no. 2146) were purchased from Cell Signaling (Danvers, MA, USA) and were diluted 1:1000. UCP1 polyclonal antibody (no. PA1-24894) and PR domain-containing 16 polyclonal antibody (PRDM16; no. PA5-20872) were purchased from Thermo Scientific (Waltham, MA, USA) and were diluted 1:1000. Anti-fatty-acid binding protein 4 antibody (FABP4; no. sc-18661) was purchased from Santa Cruz Biotechnology (Dallas, TX, USA) and was diluted 1:400. IRDye 800CW goat anti-rabbit (no. 926-32211) and IDyRye

680RD goat anti-mouse (no. 926-68070) secondary antibodies for Western blotting were purchased from LI-COR (Lincoln, NE, USA) and were diluted 1:15,000. Band density was quantified and then normalized to β -tubulin content, because the levels of β -tubulin did not differ between experimental groups.

Statistical analysis

Data are presented as means \pm SEM. The general linear model and Duncan's multiple range test (SAS Institute Inc., Cary, NC, USA) were used to analyse data and to determine the significance of differences among means of different treatments. A value of $P < 0.05$ was considered to be statistically significant.

Results

Maternal phenotype and metabolic parameters

The initial non-pregnant female body weights were similar among groups (CON 19.8 ± 0.27 g, CON+RES 20.4 ± 0.44 g, HFD 19.6 ± 0.29 g and HFD + RES 19.8 ± 0.44 g). At weaning, HFD-fed dams had higher body weight (Fig. 1A) and ratio of WAT mass to body weight (Fig. 1B) than CON and CON+RES dams. Dietary RES protected dams against HFD-induced body weight gain (Fig. 1A) and fat accumulation (Fig. 1B). Administration of RES did not change energy intake during pregnancy and lactation (Fig. 1C). Moreover, no difference was observed in maternal blood glucose concentrations (Fig. 1D). The HFD-fed dams exhibited increased serum triglyceride and insulin concentrations, whereas RES supplementation to HFD dams significantly reduced the concentrations of triglycerides and insulin. There was no difference in triglyceride and insulin concentrations between CON and CON+RES dams (Fig. 1E and F).

Resveratrol increased metabolic activity in BAT of HFD offspring at weaning

After birth, HFD-fed male offspring showed increased body weight, which was reduced by maternal RES treatment (CON 1.28 ± 0.03 g, CON+RES 1.22 ± 0.02 g, HFD 1.54 ± 0.05 g and HFD+RES 1.32 ± 0.05 g; $P < 0.01$ for CON vs. HFD, $P < 0.05$ for HFD vs. HFD+RES). Similar changes in offspring body weight were also observed at 7, 14 and 21 days (Fig. 2A), as well as in WAT mass at weaning (Fig. 2B). Adipose tissues of mice at post-natal day 21 were analysed further. Although BAT mass was unchanged, HFD offspring BAT became filled with large lipid droplets, a phenotype similar to white fat, which was recovered following RES supplementation (Fig. 2C), consistent with changes in UCP1 immunohistochemical staining (Fig. 2C). In agreement, the UCP1 protein level (Fig. 2E and F) was reduced by the HFD but recovered by maternal RES supplementation. In addition, the mRNA levels of PPAR γ coactivator-1 α (*PGC-1 α*), *PRDM16*, cell death-inducing DFFA-like effector A (*Cidea*), elongation of very long-chain fatty acids protein 3 (*Elovl3*) and cytochrome *c* oxidase subunit Vlla polypeptide 1 (*Cox7a1*) were all decreased in HFD offspring compared with other treatments (Fig. 2D). The decreased *PRDM16* protein levels in HFD offspring was largely prevented by maternal RES treatment (Fig. 2E and F). The HFD decreased AMPK α phosphorylation, with no effect on

total AMPK α (t-AMPK α), resulting in a decrease in the p-AMPK α /t-AMPK α ratio, which was recovered by RES (Fig. 2E and F). A similar pattern of changes was observed for Sirt1 (Fig. 2E and F). Together, these results provide evidence that maternal administration of RES increased the thermogenic activity of BAT in HFD offspring.

At weaning, non-fasting glucose was higher in HFD offspring than that of CON and CON+RES offspring, but maternal RES supplementation did not decrease the glucose concentration in HFD offspring (Fig. 2G). There were significant increases in serum triglyceride and insulin concentrations in HFD offspring compared with CON and CON+RES offspring, which were reduced by maternal RES treatment, but remained slightly higher than CON ($P < 0.05$; Fig. 2H and I).

Resveratrol induces brown fat-like changes in WAT of HFD offspring at weaning

Besides BAT activity, browning of WAT is another important process in adaptive thermogenesis. We investigated the effects of maternal RES supplementation on thermogenic programming in WAT, both IngWAT and EpiWAT. Maternal RES supplementation noticeably increased the abundance of multilocular adipocytes in IngWAT (Fig. 3A) and EpiWAT (Fig. 4A) of HFD offspring, which is a key characteristic of brown fat-like cells.

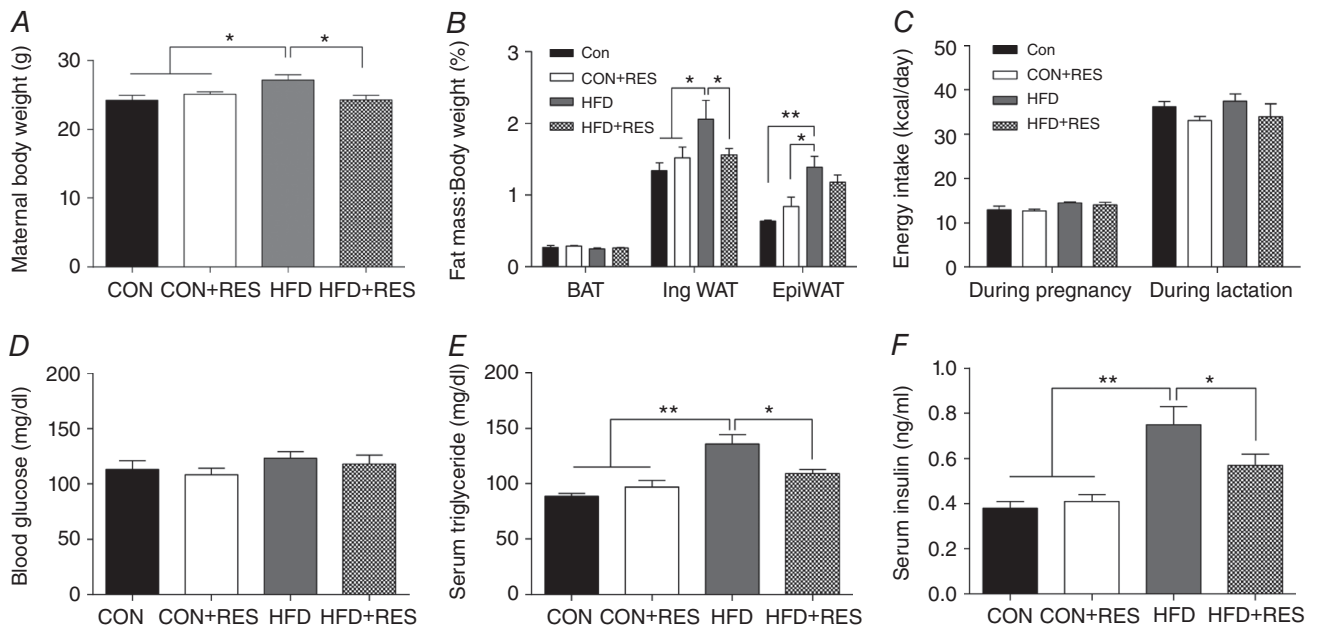


Figure 1. Maternal characteristics

A, maternal body weight at the end of lactation. B, fat index (percentage of fat pad weight relative to the whole body weight) of BAT, inguinal WAT and epididymal WAT. C, maternal energy intake during pregnancy and lactation. D–F, blood glucose (D), serum triglyceride (E) and insulin concentrations (F) at the end of lactation. * $P < 0.05$ and ** $P < 0.01$. Data are shown as means + SEM. ($n = 8$). Abbreviations: BAT, brown adipose tissue; CON, control; EpiWAT, epididymal white adipose tissue; HFD, high-fat diet; IngWAT, inguinal white adipose tissue; RES, resveratrol; WAT white adipose tissue.

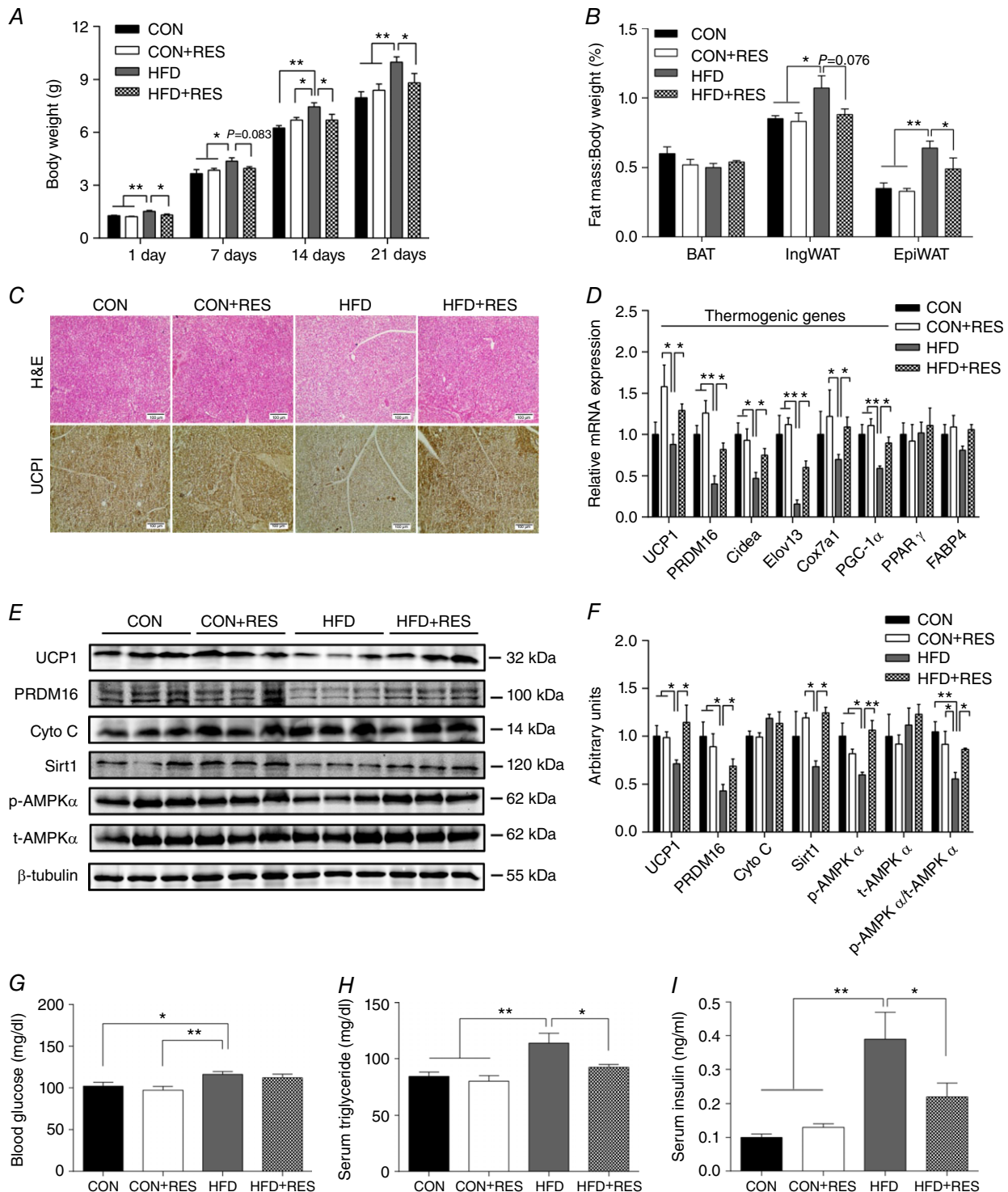


Figure 2. Maternal resveratrol supplementation increased BAT metabolic activity in male HFD offspring at weaning

A, body weight ($n = 8$). B, fat index (percentage of fat pad weight relative to the whole body weight) of BAT, inguinal WAT and epididymal WAT ($n = 8$). C, representative H&E staining and UCP1 staining for BAT sections ($n = 8$). D, mRNA expression of thermogenic genes in BAT ($n = 6$). E and F, immunoblotting analysis for thermogenic genes and AMPK α and Sirt1 ($n = 6$). G–I, blood glucose (G) and serum triglyceride (H) and insulin concentrations (I) of the offspring at weaning ($n = 8$). * $P < 0.05$ and ** $P < 0.01$. Data are shown as means + SEM. Abbreviations: AMPK α , AMP-activated protein kinase; H&E, Haematoxylin and Eosin; Sirt1, Sirtuin 1; UCP1, uncoupling protein 1; for other abbreviations, see legend to Fig. 1. [Colour figure can be viewed at wileyonlinelibrary.com]

UCP1 IHC showed enhanced UCP1 levels in IngWAT (Fig. 3A) and EpiWAT (Fig. 4A) of HFD+RES offspring compared with HFD offspring. Moreover, maternal RES administration decreased the adipocyte diameter in IngWAT (Fig. 3A and B) and EpiWAT (Fig. 4A and B).

Consistent with the changes in UCP1 immunostaining, *UCP1* mRNA and UCP1 protein expression were reduced in IngWAT (Fig. 3C, E and F) and EpiWAT (Fig. 4C, E and F) of HFD mice. This reduction was prevented by RES. Correspondingly, maternal RES supplementation

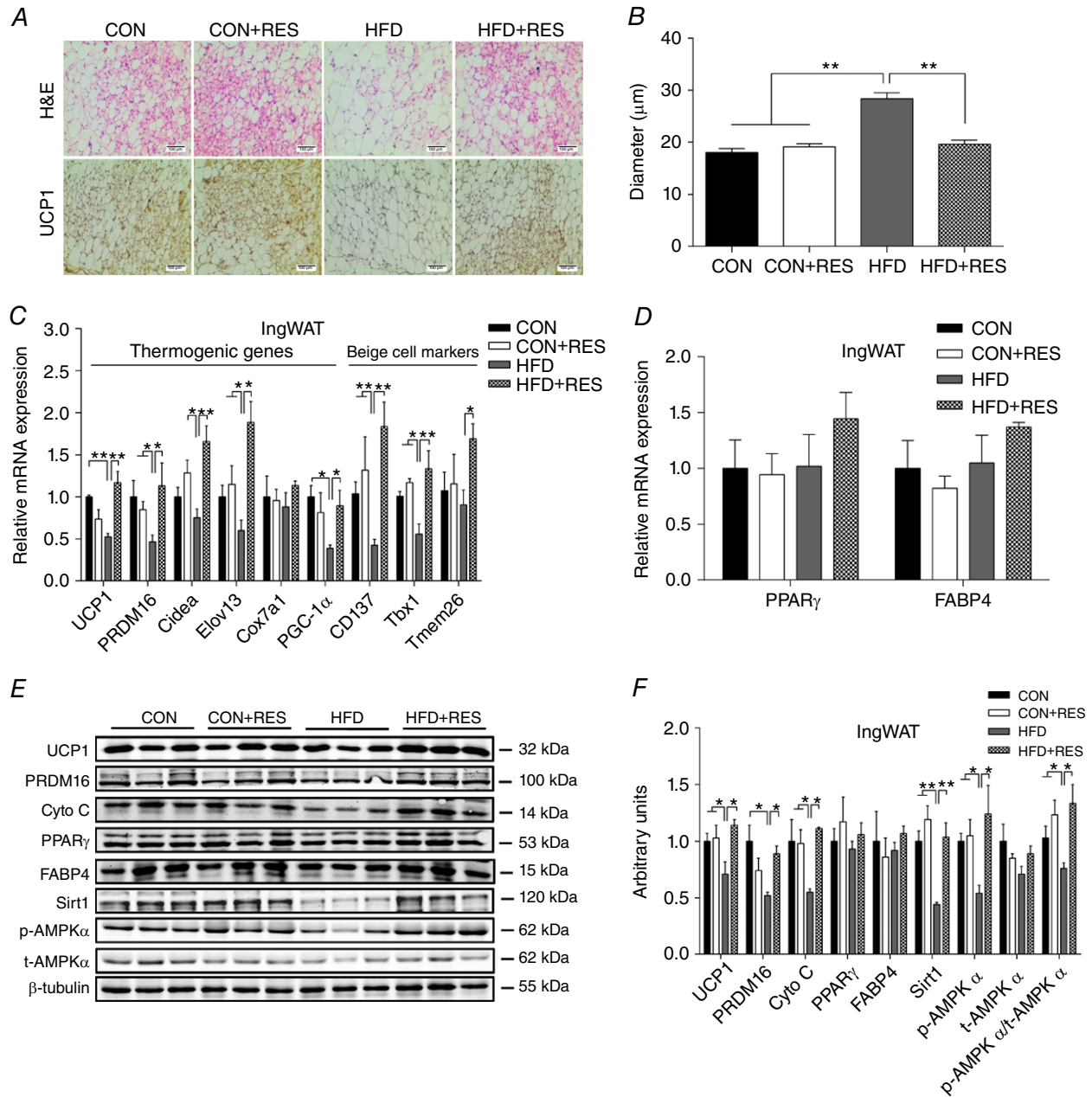


Figure 3. Maternal resveratrol supplementation induced brown fat-like changes in IngWAT of HFD offspring at weaning

A and B, representative H&E staining and UCP1 staining (A) and average adipocyte diameters (B) in IngWAT ($n = 8$). C, mRNA expression of thermogenic genes and beige adipocyte-selective markers in IngWAT ($n = 6$). D, mRNA expression of *PPARγ* and *FABP4* in IngWAT ($n = 6$). E and F, immunoblotting analysis of thermogenic genes and AMPKα and Sirt1 in IngWAT ($n = 6$). * $P < 0.05$ and ** $P < 0.01$. Data are shown as means + SEM. Abbreviations: AMPKα, AMP-activated protein kinase; FABP4, fatty-acid binding protein 4; H&E, Haematoxylin and Eosin; PPARγ, peroxisomal proliferator-activated receptor γ; Sirt1, Sirtuin 1; UCP1, uncoupling protein 1; for other abbreviations, see legend to Fig. 1. [Colour figure can be viewed at wileyonlinelibrary.com]

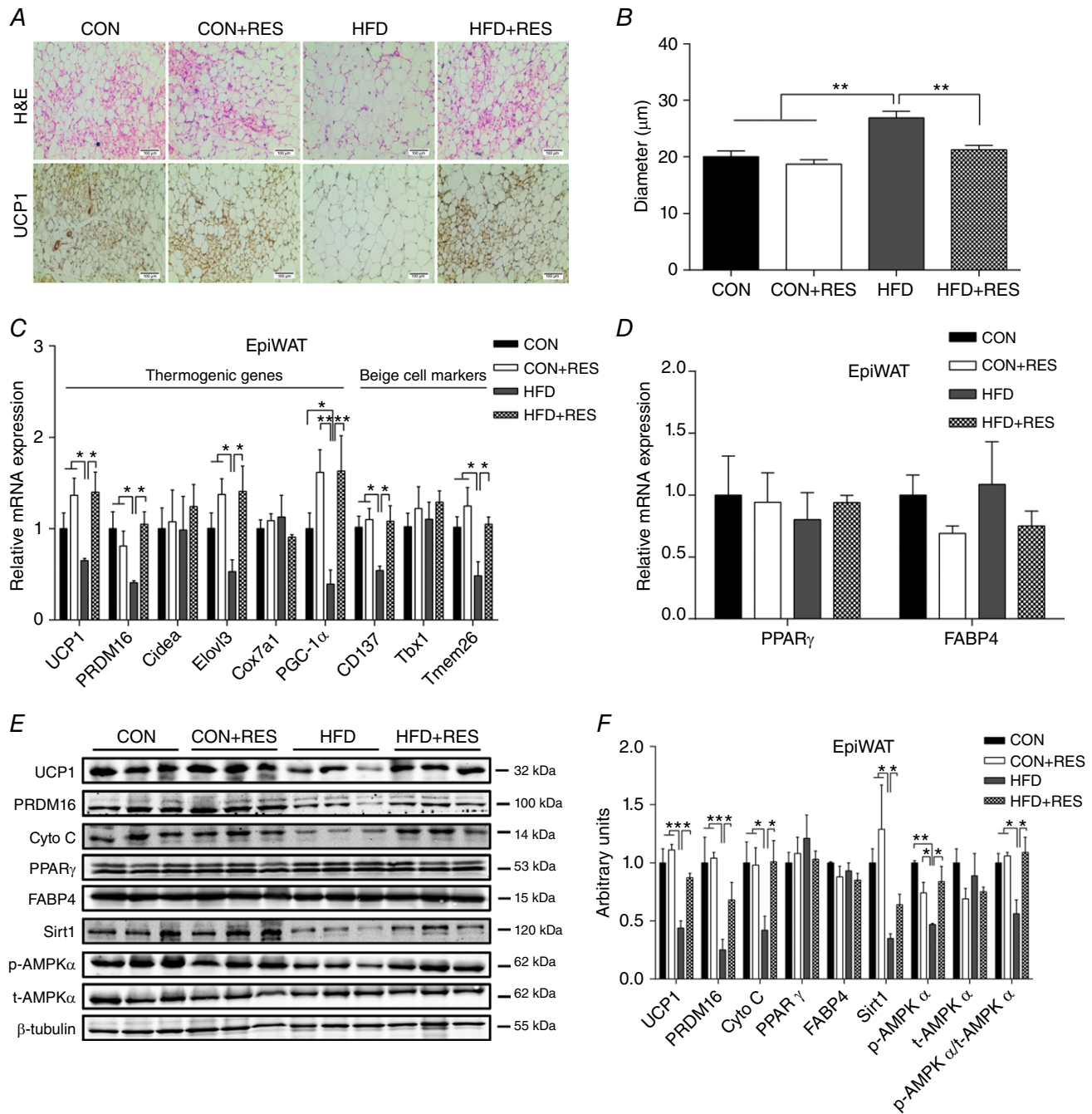


Figure 4. Maternal resveratrol supplementation induced brown fat-like changes in EpiWAT of HFD offspring at weaning

A and B, representative H&E staining and UCP1 staining (A) and average adipocyte diameters (B) in EpiWAT ($n = 8$). C, mRNA expression of thermogenic genes and beige adipocyte-selective markers in EpiWAT ($n = 6$). D, mRNA expression of *PPAR γ* and *FABP4* in EpiWAT ($n = 6$). E and F, immunoblotting analysis of thermogenic genes and AMPK α and Sirt1 in EpiWAT ($n = 6$). * $P < 0.05$ and ** $P < 0.01$. Data are shown as means + SEM. Abbreviations: AMPK α , AMP-activated protein kinase; FABP4, fatty-acid binding protein 4; H&E, Haematoxylin and Eosin; PPAR γ , peroxisomal proliferator-activated receptor γ ; Sirt1, Sirtuin 1; UCP1, uncoupling protein 1; for other abbreviations, see legend to Fig. 1. [Colour figure can be viewed at wileyonlinelibrary.com]

increased the expression of a series of thermogenic genes in IngWAT of HFD offspring, including *PRDM16*, *Cidea*, *Elovl3* and *PGC-1 α* (Fig. 3C). A similar pattern of thermogenic gene expression was noticed in EpiWAT (Fig. 4C). Moreover, maternal RES treatment also induced the mRNA expression of beige adipocyte-selective markers in IngWAT (Fig. 3C) and EpiWAT (Fig. 4C) of HFD offspring, including cluster of differentiation 137 (*CD137*), T-box 1 (*Tbx1*) and transmembrane protein 26 (*Tmem26*). In addition, the decreased PRDM16 protein levels in IngWAT (Fig. 3E and F) and EpiWAT (Fig. 4E and F) of HFD offspring were increased by maternal RES treatment. Compared with CON and CON+RES groups, HFD decreased the protein levels of cytochrome *c* (Cyto C), an important component of mitochondrial respiratory chain, in IngWAT (Fig. 3E and F) and EpiWAT (Fig. 4E and F). Resveratrol treatment of dams fed HFD increased Cyto C protein levels, accompanied by u-regulated expression of p-AMPK α and Sirt1 in IngWAT (Fig. 3E and F) and

EpiWAT (Fig. 4E and F), indicating that RES activated the AMPK/Sirt1 pathway. We found that maternal RES supplementation had no effect on the expression of PPAR γ and FABP4 at both mRNA and protein levels in IngWAT (Fig. 3D–F) and EpiWAT (Fig. 4D–F). These results suggested that maternal RES drives browning of WAT during early development.

Resveratrol reduces adiposity and improves insulin sensitivity in adult offspring challenged with HFD

To determine whether the increased brown and beige adipogenesis during the early developmental stage could exert long-time benefits in preventing diet-induced obesity in adulthood, we examined the body composition and metabolic characteristics of offspring mice at 3 months of age. The HFD offspring showed a marked increase in body weight (Fig. 5A) and WAT mass (Fig. 5B) compared with CON and CON+RES. Maternal RES

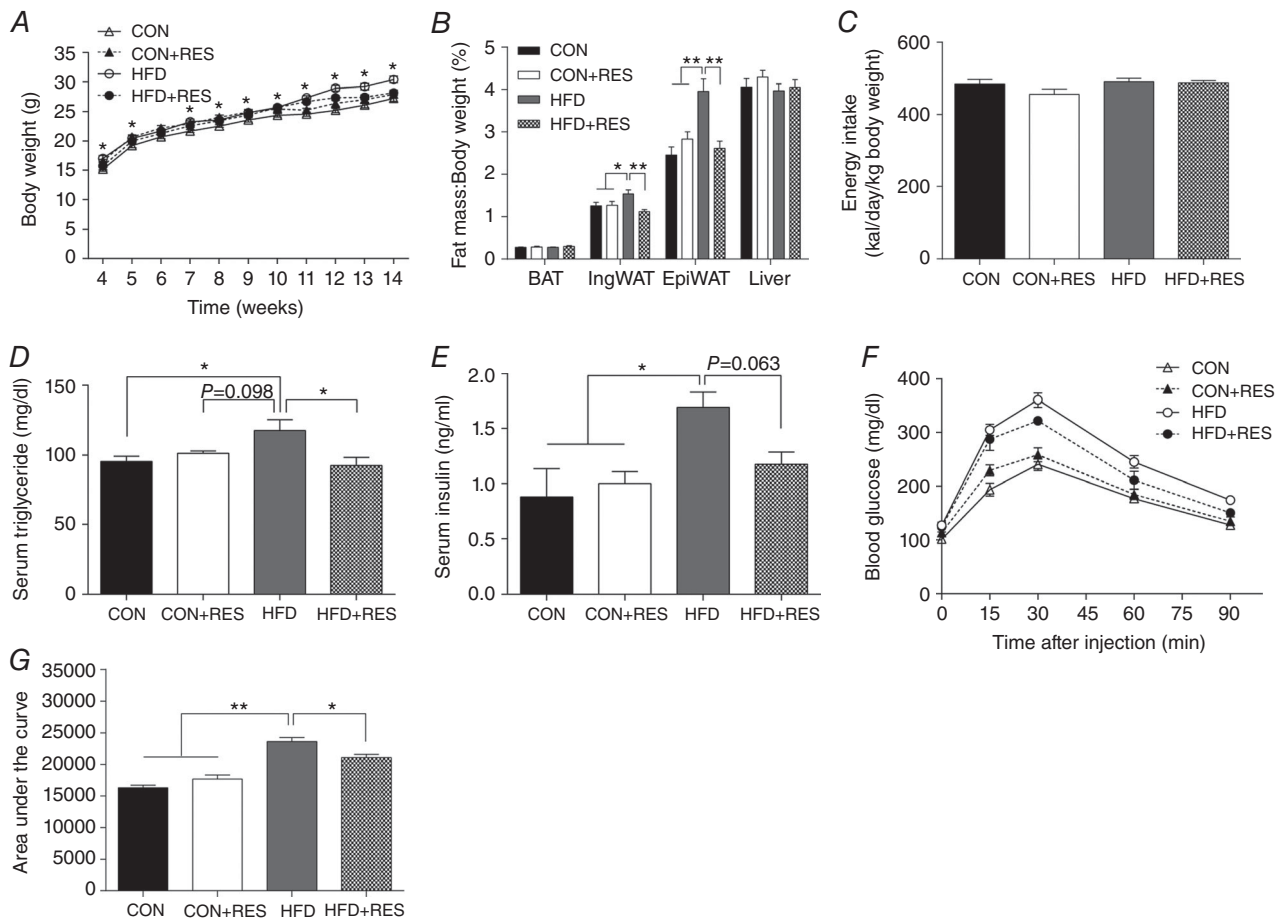


Figure 5. Maternal resveratrol supplementation reduced body weight gain and adiposity and improved insulin sensitivity in 14-week-old offspring challenged with HFD

A, offspring body weight (n = 8–10). B, ratio of adipose tissue and liver weights to body weights (n = 8–10). C, offspring energy intake (n = 8–10). D, serum triglyceride concentrations (n = 6). E, serum insulin concentrations (n = 6). F and G, glucose tolerance test and area under the curve (n = 6). *P < 0.05 and **P < 0.01. Data are expressed as means \pm SEM. For abbreviations, see legend to Fig. 1.

treatment during pregnancy and lactation protected adult offspring against HFD-induced body weight gain (Fig. 5A) and fat accumulation (Fig. 5B). These responses occurred without differences in energy intake (Fig. 5C). Compared with CON and CON+RES offspring, HFD offspring showed higher serum triglyceride and insulin concentrations, which were attenuated by maternal RES treatment to a level comparable with CON (Fig. 5D and E). Maternal RES supplementation improved glucose tolerance in HFD offspring (Fig. 5F and G). These data suggested that maternal RES supplementation prevented diet-induced obesity and increased insulin sensitivity in adulthood.

Resveratrol increases whole-body energy expenditure in adult offspring challenged with HFD

To explore why maternal RES feeding protected against diet-induced obesity in adult offspring, we measured offspring whole-body energy expenditure. Compared with CON and CON+RES offspring, HFD offspring showed markedly decreased oxygen consumption during light and dark phases, which was prevented by maternal RES treatment (Fig. 6A and B). There was no difference in CO₂ production among groups (Fig. 6C and D). As a result, the increased RER in HFD offspring tended to be decreased by maternal RES treatment (Fig. 6E and F), suggesting that a higher ratio of lipids was being oxidized,

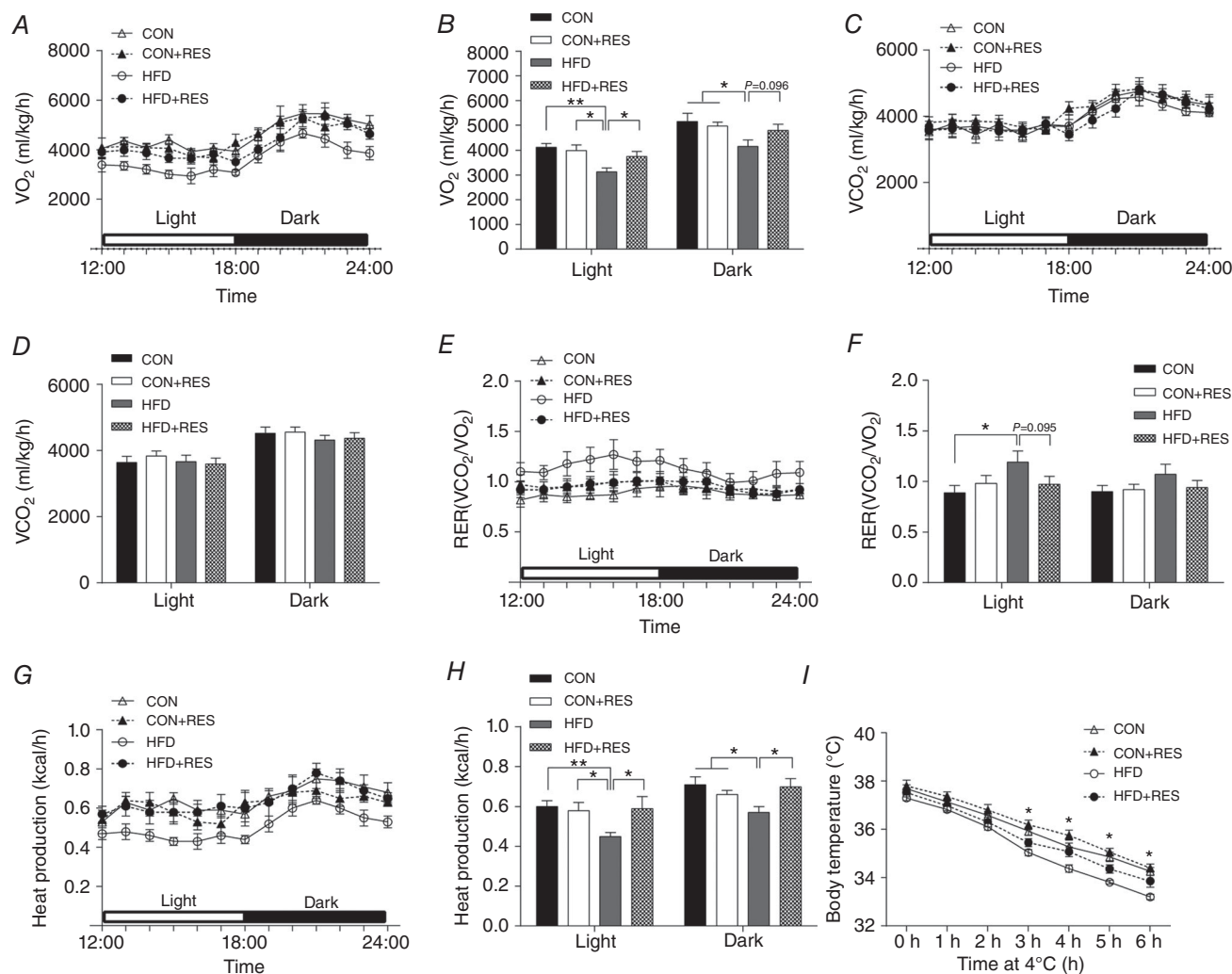


Figure 6. Maternal resveratrol supplementation increased energy expenditure in 14-week-old offspring under HFD challenge

A and B, $\dot{V}O_2$ during a 6 h light–6 h dark cycle measured in a metabolic cage (A) and the average values (B). C and D, $\dot{V}CO_2$ during a 6 h light–6 h dark cycle (C) and the average values (D). E and F, the values of RER (ratio of $\dot{V}CO_2$ to $\dot{V}O_2$) were calculated from metabolic chamber data during a 6 h light–6 h dark cycle. G and H, heat production during a 6 h light–6 h dark cycle (G) and the average values (H). I, acute cold tolerance test. * $P < 0.05$ and ** $P < 0.01$. Data are expressed as means \pm SEM ($n = 6$). Abbreviations: RER, respiratory exchange rate; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}O_2$, oxygen consumption rate; for other abbreviations, see legend to Fig. 1.

consistent with the decreased circulating triglyceride concentrations in HFD+RES offspring. Furthermore, in accordance with oxygen consumption, the decreased heat production in HFD offspring was prevented by maternal RES treatment (Fig. 6G and H). Moreover, HFD+RES offspring had a higher body temperature than HFD offspring during the cold challenge, indicating that maternal RES treatment enhanced the capacity for adaptive thermogenesis (Fig. 6I). Taken together, these results showed that maternal RES supplementation increased metabolic activity in HFD adult offspring.

Resveratrol promotes BAT function and IngWAT browning in adult offspring challenged with HFD

To explain the enhanced energy expenditure in HFD+RES offspring, we examined whether BAT function and WAT thermogenic programme are elevated in these mice. Haematoxylin and Eosin staining results revealed that the average adipocyte diameters in WAT of HFD+RES offspring were smaller than those of HFD offspring (Fig. 7A). Large adipocytes were most abundant in WAT of HFD offspring, whereas maternal RES treatment attenuated this HFD-induced effect (Fig. 7B and C). In addition, histological analysis also indicated that HFD+RES offspring displayed fewer lipid deposits and greater UCP1 expression in BAT compared with HFD offspring (Fig. 7A). Similar to their BAT, IngWAT of HFD+RES offspring mice also displayed more beige adipocytes and higher UCP1-positive areas than those of HFD offspring mice (Fig. 7A and D). Consistent with these findings, maternal RES supplementation increased UCP1 protein expression in BAT and IngWAT of adult HFD offspring (Fig. 7E and F). However, the morphological changes (Fig. 7A and D) and expressional alteration of UCP1 (Fig. 7G) were not observed in EpiWAT of HFD+RES offspring. These data suggested that maternal RES supplementation increased BAT function and induced beige adipocyte development in IngWAT of adult offspring.

Discussion

It has been well established that maternal obesity and high energy intake impair fetal development, with subsequent long-term negative effects on offspring health. Several studies have shown that maternal polyphenol supplementation during the fetal and lactation stages improves offspring development, reducing body fat accumulation and metabolic disorders, such as hypertension, insulin resistance and inflammation, in adult life (Dolinoy *et al.* 2006; Resende *et al.* 2013; Wu *et al.* 2014). However, the mechanistic links remain to be established.

In the present study, we used the diet-induced obesity model to test the effect of RES on offspring adipose

development. The body weight of HFD neonates was higher than that of CON neonates at postnatal day 1, similar to macrosomia of the babies of obese women, and maternal resveratrol treatment reduced the body weight gain of HFD neonates. The biological effects of RES on fetal development should be attributable to both maternal and direct fetal effects. Resveratrol can cross the placenta and affect the fetus directly (Bourque *et al.* 2012). In addition, RES also alters maternal metabolism to change the intra-uterine and early postnatal nutritional environments, which in turn affects the early programming of the offspring (Sun *et al.* 2012). At the end of lactation, RES supplementation did not significantly alter blood glucose concentrations in HFD dams and their weaned offspring; findings which are similar to previous reports demonstrating that maternal polyphenol administration had no significant effect on blood glucose in HFD-fed dams in gestation or lactation (Roberts *et al.* 2014; Wu *et al.* 2014). Moreover, the HFD dams had higher body weight, WAT mass and circulating concentrations of triglycerides and insulin compared with CON dams, which was recovered by maternal RES treatment, suggesting a positive effect of RES on maternal metabolic homeostasis.

Two forms of adipose tissue exist, the white and brown adipose tissues. While WAT is responsible for obesity and metabolic dysfunction, BAT burns fatty acids and glucose to generate heat (Virtanen *et al.* 2009). Brown adipose tissue is capable of producing 300 times more heat than other tissues per unit mass (Symonds, 2013). Thus, the recent discovery of BAT in adult humans provides a promising therapeutic target against obesity and diabetes (Kozak *et al.* 2010; Peirce *et al.* 2014). In addition, beige adipocytes have recently been identified in WAT (Harms & Seale, 2013). Importantly, beige adipocytes are inducible, a process termed 'browning of WAT' (Wu *et al.* 2012). In adult animals, RES could reduce body weight gain and adiposity via inhibition of white adipogenesis (Lagouge *et al.* 2006; Kim *et al.* 2011) and induction of browning of WAT (Wang *et al.* 2015). To date, however, no study has assessed the effect of maternal RES administration on adipocyte browning and thermogenesis in offspring born to maternal HFD mothers. In the present study, we found that maternal HFD intake during pregnancy and lactation impaired offspring BAT and beige adipocyte development at weaning and had lasting effects on offspring metabolic health. Importantly, RES treatment of HFD dams not only enhanced BAT activity and beige adipocyte formation and the associated thermogenic programme in offspring, but also had persistent beneficial effects in preventing HFD-induced obesity and metabolic disorders in adulthood. To our knowledge, this is the first report showing promotion of BAT/beige adipogenesis and thermogenic function attributable to RES supplementation of HFD mothers.

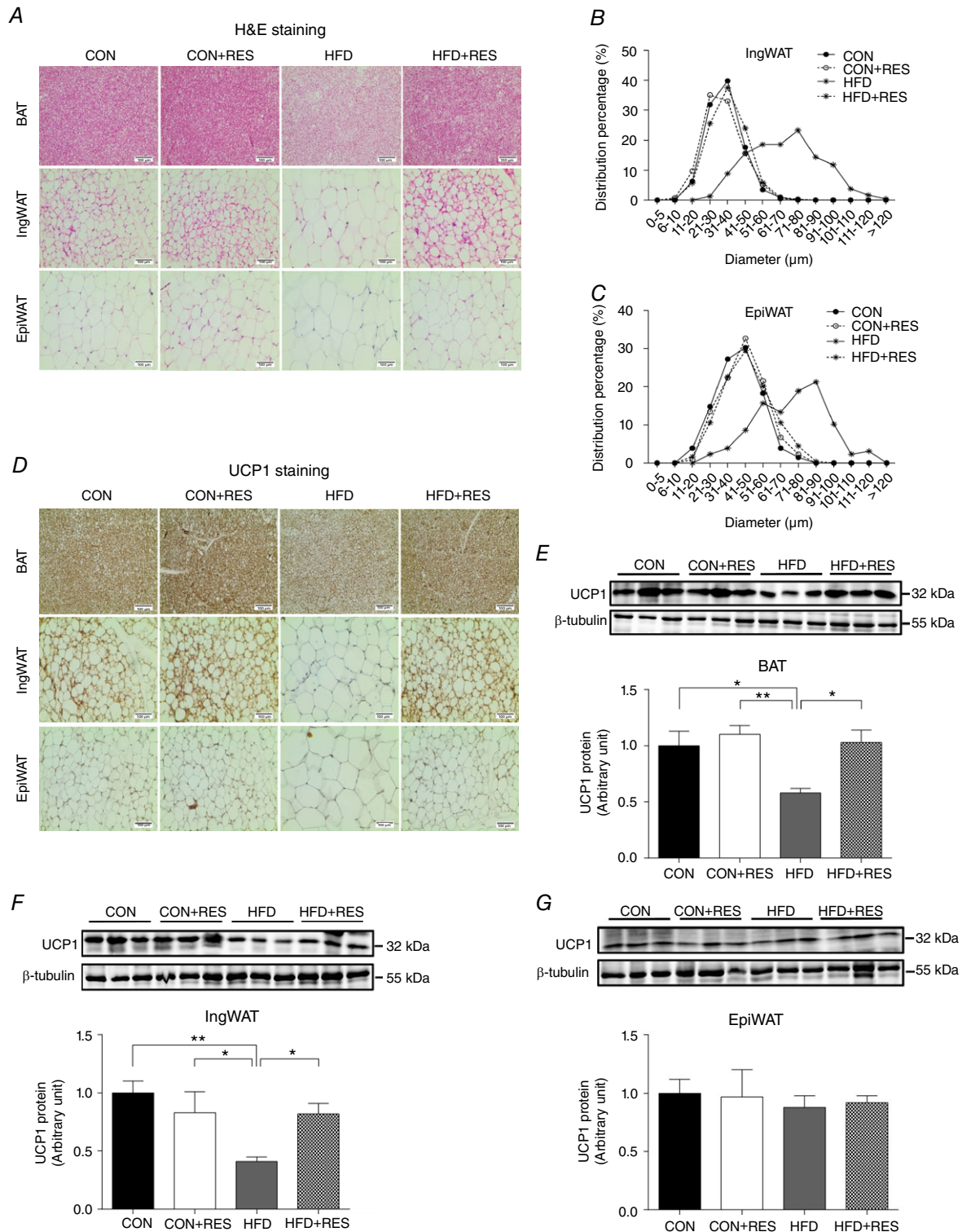


Figure 7. Maternal resveratrol supplementation enhanced BAT function and IngWAT thermogenic programme in 14-week-old offspring challenged with HFD

A, representative H&E staining in sections of BAT and WAT ($n = 8$). B and C, percentage distribution of adipocyte diameters of IngWAT (B) and EpiWAT (C) ($n = 8$). D, representative UCP1 staining in sections of BAT and WAT ($n = 8$). E–G, immunoblotting analysis for UCP1 in BAT (E), IngWAT (F) and EpiWAT (G) ($n = 6$). * $P < 0.05$ and ** $P < 0.01$. Data are shown as means \pm SEM. Abbreviations: H&E, Haematoxylin and Eosin; UCP1, uncoupling protein 1; for other abbreviations, see legend to Fig. 1. [Colour figure can be viewed at wileyonlinelibrary.com]

The development and thermogenic function of brown/beige adipocytes are regulated by a complex network of hormones and signalling pathways. PRDM16 is a crucial transcription factor that drives brown adipogenic gene expression and thermogenesis (Hondares *et al.* 2011). In our previous studies, we found that RES activates AMPK and Sirt1 (Wang *et al.* 2015), and we also found that AMPK promotes PRDM16 expression (Yang *et al.* 2016). Consistent with these findings, in the present study, maternal RES treatment increased the expression of *PRDM16* and other thermogenic genes in HFD+RES offspring. These findings demonstrated that maternal RES supplementation promotes thermogenic activity of BAT and beige adipocytes in offspring at weaning. As a consequence, the increase in body weight gain and WAT mass of HFD offspring was prevented by RES supplementation. AMPK and Sirt1, two important energy sensors, act together with PGC-1 α to regulate energy homeostasis in response to environmental and nutritional stimuli (Cantó & Auwerx, 2009). We found that maternal RES supplementation increased phosphorylated AMPK α levels and Sirt1 protein contents in BAT and WAT of HFD offspring, consistent with previous reports showing that RES-induced biological effects are mediated by the AMPK/Sirt1 pathway (Kitada *et al.* 2011; Price *et al.* 2012), and also in adult mice, where grape seed procyanidins (Crescenti *et al.* 2015) and azuki (Mukai *et al.* 2013) activate the AMPK/Sirt1 pathway.

The development of BAT mainly occurs during the late fetal stage, and its thermogenic function is crucial for an adequate neonatal response to prevent hypothermia. During fetal development, myogenic factor (MYF)⁵⁺ cells in the future BAT depots start to express *Prdm16*, which initiates brown adipogenic gene cascades in myogenic precursor cells (Seale *et al.* 2008). In addition, based on lineage tracing, a portion of brown adipocytes in BAT are developed from non-myogenic PDGFR α ⁺ progenitor cells (Tran *et al.* 2012; Peirce *et al.* 2014), whereas most of the beige adipocytes in WAT are derived from PDGFR α ⁺ progenitor cells (McDonald *et al.* 2015; Sharp *et al.* 2015; Townsend & Tseng, 2015). The density of progenitor cells varies among different fat depots. The subcutaneous fat has a high density of progenitor cells and high expansion capacity, whereas that of EpiWAT is limited (Liang *et al.* 2016). The low density of progenitor cells in EpiWAT limits *de novo* formation of beige adipocytes, which might explain the lack of changes in EpiWAT beige adipogenesis of offspring born to HFD+RES mothers, because new beige adipocytes are primarily derived from progenitor cells (Wang *et al.* 2013; Lee *et al.* 2015).

In conclusion, we have demonstrated that RES supplementation of mice fed a HFD during pregnancy and lactation promotes a thermogenic programme in BAT and WAT, and induces beige adipocyte development of

WAT in their male offspring. Furthermore, the enhanced BAT function and browning of WAT increased energy expenditure, protecting offspring against HFD-induced obesity and insulin resistance in adulthood. Thus, we propose that maternal intervention with RES throughout pregnancy and lactation has promise as an intervention in the setting of maternal obesity by inducing persistent beneficial programming effects on the BAT/beige adipocyte function and thermogenesis in male offspring, with lasting effects on offspring metabolic health.

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Additional information

Competing interests

The authors declare no conflict of interest.

Author contributions

T.Z. and M.D. conceived the project, designed the experiments and wrote the manuscript. T.Z., D.C., Q.Y. and B.W. researched

data. T.Z., Q.Y. and B.W. performed the experiments. D.C., M.-J.Z. and P.W.N. contributed to the discussion and reviewed and edited the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This work was supported by grants from the National Institutes of Health (R01-HD067449 and R21-AG049976) and a grant from the National Processed Raspberry Council to M.D., the National Basic Research Program of China (2012CB124701) to D.C. and a scholarship from the China Scholarship Council to T.Z.