Original Research

Prenatal and early postnatal exposure to high-saturated-fat diet represses Wnt signaling and myogenic genes in offspring rats

Ke-Feng Yang[1,2,3,4], Xiu-Hua Shen[1,2] and Wei Cai[1,2,3,4]

[1]Clinical Nutrition Center, Xin Hua Hospital, Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, Shanghai 200092; [2]Department of Nutrition, Shanghai Jiao Tong University School of Medicine, Shanghai 200025; [3]Shanghai Institute for Pediatric Research; [4]Shanghai Key Laboratory of Pediatric Gastroenterology and Nutrition, 1665 Kongjiang Road, Shanghai 200092, China
Corresponding author: Wei Cai. Email: caiw204@yahoo.com.cn

Abstract

The prenatal and early postnatal period is a key developmental window for nutrition status, and high-fat exposure in this period has been shown to be associated with type 2 diabetes, obesity and other features of metabolic disorders later in life. The present study was designed to investigate the underlying molecular mechanisms and role of relative genes involved in this process. We investigated the impact of prenatal and early postnatal exposure to a high-saturated-fat diet on the regulation of the Wnt signaling pathway and myogenic genes in skeletal muscle of rat offspring as well as the serum and muscle physiological outcomes. Timed-pregnant Sprague-Dawley rats were fed either a control (C, 16% kcal fat) or high-saturated-fat diet (HF, 45% kcal fat) throughout gestation and lactation. After weaning, female offspring were fed a control diet to generate two offspring groups: control diet-fed offspring of control diet-fed dams (C/C) and control diet-fed offspring of HF diet-fed dams (HF/C). The serum glucose of the HF/C offspring (5.58 ± 0.26 mmol/L) was significantly higher than that of C/C offspring (4.97 ± 0.28 mmol/L), and the Homeostasis Model Assessment-Insulin Resistance of HF/C offspring (2.00 ± 0.11) was also significantly higher when compared with C/C (1.84 ± 0.09). Furthermore, HF/C offspring presented excessive intramuscular fat accumulation (1.8-fold, $P < 0.05$) and decreased muscle glycogen (1.3-fold, $P < 0.05$), as well as impairment of muscle development at the age of 12 weeks. Meanwhile, we observed the repression of Wnt/β-catenin signaling and myogenic genes in HF/C offspring. The present study indicates that prenatal and early postnatal exposure to a high-saturated-fat diet suppresses the development of skeletal muscle and myogenic genes via Wnt/β-catenin signaling, and the inappropriate muscle development could potentially contribute to the predisposition of offspring to develop metabolic-syndrome-like phenotype in adulthood.

Keywords: perinatal stress, thrifty phenotype hypothesis, myogenesis, high-fat diet, Wnt signaling, metabolic syndrome

Experimental Biology and Medicine 2012; **237**: 912–918. DOI: 10.1258/ebm.2012.011395

Introduction

The high-fat dietary pattern is common throughout the world, and is believed to increase the morbidity of diet-related chronic diseases such as diabetes and obesity.[1–4] Early-life (prenatal and early postnatal period) is a key developmental window to nutrition exposure, and excessive nutritional supply during this period has been shown to be associated with type 2 diabetes, obesity and other features of metabolic disorders, such effects being independent of adult nutritional environment factors.[5,6] This association has been explained by the 'fetal programming hypothesis' or 'fetal origins of adult disease hypothesis', which indicates that nutritional influences during early-life development lead to fetal adaptations that can cause structural, physiological and metabolic changes.[7–9]

Animal studies demonstrated that the rat offspring whose mothers were fed a high-fat diet exhibited elevated whole body adiposity, reduced insulin sensitivity, poor glycemic control, elevated tissue triglyceride content and increased blood pressure.[10–12] A study in humans has shown that children of obese mothers demonstrated early indicators of obesity predisposition, including increased body mass index, elevated body fat content and reduced energy expenditure.[13] Although the physiological consequences of a high-fat diet in early life have been widely studied, the underlying molecular mechanisms remain unclear and the role of relative genes has not yet been elucidated.

In humans, skeletal muscle composes 40–50% of the body weight of adults, making it quantitatively the most important tissue for glucose and fatty acid metabolism. *In vivo*

studies have also demonstrated that skeletal muscle is the principal site of glucose uptake, accounting for about 85% of glucose disposal.[14] As the Wnt signaling pathway plays a critical role in muscle development, and also participates in the development of metabolic syndrome,[15,16] we therefore propose that prenatal and early postnatal exposure to high-saturated-fat (HF) influences the Wnt signaling pathway in skeletal muscle, and subsequently induces alterations of the downstream target myogenic genes, which could then be responsible for the mechanism underlying physiological consequences of prenatal and early postnatal exposure to HF. In the present study, we fed a high-saturated-fat diet to pregnant dams during gestation and lactation to investigate Wnt signaling and myogenic genes in offspring; we also examined the potential connections between maternal exposure to HF and metabolic outcomes in adult offspring, including body weight, glucose metabolism, intramuscular fat and muscle development.

Materials and methods

Animals and treatment

Timed-pregnant Sprague-Dawley rats (80 days old; Shanghai Slac Laboratory Animal Inc., Shanghai, China) were at day 1 of gestation, determined by taking vaginal swabs of females co-housed with males, and randomly assigned to two groups (each group $n = 6$): control (20% protein, 16% fat and 64% carbohydrate, by calories) or HF diet (20% protein, 45% fat and 35% carbohydrate, by calories) throughout gestation and lactation periods. The HF diet and control diet were modified according to the AIN-93 diet formula (Table 1). The fat in the HF group was mainly composed of lard, which is rich in saturated fatty acids fat (saturated fatty acids provide 18% of calories in the HF group versus 5% for the control group). The essential fatty acids of both diets meet the requirement for growth and development of dams and pups.[17] Animals were individually housed in standard polycarbonate cages and maintained in a humidity- and temperature-controlled room on a 12-h light–dark cycle with access to water and chow *ad libitum*. Twenty-four hours after birth, litter sizes were standardized to 10 pups to minimize variation in offspring nutrition status during suckling. Female offspring were fed a control (C) diet after weaning at age three weeks to generate two offspring groups: control diet-fed offspring of control diet-fed dams (C/C) and control diet-fed offspring of HF diet-fed dams (HF/C). Rat offspring were sacrificed at two time points: the first was at the age of three weeks ($n = 10$ per group) and the second was when they were 12 weeks old ($n = 10$ per group). The animal protocol for this study was approved by the Animal Care and Welfare Committee of Shanghai Jiao Tong University, School of Medicine.

Table 1 Diet composition

	Control (C)	High fat (HF)
Macronutrients (% of total kcal)		
Protein	20	20
Carbohydrate	64	35
Fat	16	45
Ingredient (g/kg)		
Casein	200	200
L-Cystine	3	3
Corn starch	437.2	72.8
Maltodextrin	100	100
Sucrose	102	172
Cellulose	50	50
Soybean oil	25	25
Lard	47	177.5
Mineral mix*	35	35
Vitamin mix†	10	10
Choline	2	2

*Mineral mix (AIN-93): (Research Diets) product no. S10026 for rodent
†Vitamin mix (AIN-93): (Research Diets) product no. V10001 for rodent

Blood sample collection and analysis

Blood samples were collected from the heart after overnight fasting. Serum was collected after centrifugation at 3000 *g* for 15 min and stored at −80°C until subsequent analysis. Serum aliquots were used to measure serum glucose by the glucose oxidase method (Rongsheng Biotechnology Inc., Shanghai, China). Serum insulin was analyzed with a radioimmunoassay kit (RI-13K; Linco Research, St Charles, MI, USA). The insulin resistance index was estimated by the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR). Taking the natural log of HOMA-IR yields a normally distributed variable for statistical analysis. Serum triglyceride and cholesterol concentrations were measured enzymatically using an automatic biochemistry analyzer (Hitachi 7600, Tokyo, Japan).

Muscle histology and analysis

The gastrocnemius muscle of both hind legs was dissected out, trimmed of fat and connective tissues, and weighed. One was snap-frozen in liquid nitrogen, and stored at −80°C until subsequent analysis. The other was frozen in isopentane cooled in liquid nitrogen and then processed for sectioning by cryostat (Leica CM1900; Nussloch, Germany) after being embedded in OCT compound (Sakura Finetek USA, Torrance, CA, USA). Sections (10 μm) were stained with hematoxylin and eosin (H&E) or Oil Red O (Sigma Chemical Co., St Louis, MO, USA), and then photographed using a fluorescence microscope (Nikon Eclipse80i, Tokyo, Japan). Five fields per section and five sections per sample were randomly selected for quantification of muscle fiber area using NIS-Elements AR software (Nikon Inc.).

Muscle triglyceride and glycogen analysis

Gastrocnemius muscle samples were dissected free of inner and outer adipose tissue, connective tissue and blood vessels by microdissection under a stereo microscope as previously described.[18] Samples were then rinsed thoroughly in saline solution to remove all lipid droplets. Intramuscular triglyceride (TG) content was assayed using a method described previously.[19] Briefly, approximately 0.1 g of frozen tissue was homogenized in chloroform/methanol (1 mL, 2:1 vol/vol) over ice. Homogenates were

shaken overnight before adding 0.6% NaCl, and then the samples were centrifuged at 1500 *g* for 10 min to separate the phases. The TG-containing organic layer was separated and air-dried. The isolated lipids were re-suspended in 250 μL ethanol and then determined spectrophotometrically. Muscle glycogen content was measured spectrophotometrically by using a commercially available assay kit (Nanjing Jianchen Bioengenieering Inc., Nanjin, China).

Real-time quantitative reverse transcription polymerase chain reaction

Frozen muscle samples were ground with a mortar and pestle in liquid nitrogen, and total RNA was isolated with TRI reagent (Sigma). All purified RNA samples exhibited A_{260}/A_{280} ratios $\geq$1.6. A high-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) was used for reverse transcription of 2 μg of total RNA in a 20 μL reaction volume. The entire procedure was performed in a DNA 2720 Thermal Cycler (Applied Biosystems). The samples were heated at 37°C for two hours for reverse transcription followed by 85°C for five seconds to inactivate reverse transcriptase and terminate the reaction. To measure the relative amount of mRNA for our genes of interest, cDNA samples were analyzed in a 96-well plate using a 7300 Real-Time PCR System (Applied Biosystems) and iTag SYBR Green Supermix with ROX (Bio-Rad, Hercules, CA, USA). The reactions were activated at 95°C for 10 min accompanied by 35 cycles of 95°C for 15 s and 60°C for one minute. The ribosomal protein L7a mRNA level was also measured for the normalization of data. After polymerase chain reaction (PCR), a dissociation curve was generated by a stepwise increase of the temperature from 55 to 95°C to ensure that a unique product was amplified in the reaction. Primers for quantitative PCR were designed using Vector NTI software (InforMax Inc., Frederick, MD, USA) as follows: Wnt1, (+1663) CCCCGTGACCTCTCTGTGTATCAC and (+1742) TGAAGCCCAGGTGTGGTGGTT; Wnt3a, (+210) CAAG GCGGGCATCCAAGAGT and (+281) CTGTTGCTGACAG TGGTGCAGTTC; Wnt5a, (+1256) GCGTGGCTATGACC AGTTTAAGA and (+1357) TCCACAATCTCCGTGCAC TTT.

Nuclear extraction and Western blotting

Frozen muscle samples (200 mg) were ground in liquid nitrogen and then washed twice with ice-cold phosphate-buffered saline and pelleted by centrifugation at 1600 rpm. Samples were lysed with fresh lysis buffer (10 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES] pH 7.9, 10 mmol/L sodium chloride, 1.5 mmol/L magnesium chloride, 1 mmol/L ethylene glycol bis(2-aminoethyl) tetraacetic acid, 0.2 mol/L sucrose, 1 mmol/L dithiothreitol, 1 mmol/L phenylmethylsulfonyl fluoride, 1× protease inhibitor cocktail, 4× phosphatase inhibitor cocktail 1, 4× phosphatase inhibitor cocktail 2, 0.5% NP-40) on ice for five minutes and centrifuged again. Lysis of the re-suspended pellet was repeated twice to collect crude nuclei. Nuclei were sonicated on ice. Nuclear protein extracts were then collected from the supernatants after centrifugation at 10,000 *g*. The Lowry assay was used to determine protein content, and samples containing 40 μg of protein were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrotransfer onto a polyvinylidene fluoride membrane (0.2 μm; Bio-Rad) using a wet transfer protocol, 10% milk in Tris-buffered saline/Tween-20 (30 mmol/L Tris base pH 7.6, 200 mmol/L NaCl and 0.1% Tween 20) was used to block the membrane for one hour at room temperature and then the membrane was incubated with 1:1000 rabbit polyclonal antibody against β-catenin (#9587; Cell Signaling Technology, Danvers, MA, USA), 1:2000 Mouse Anti-MyoD1 Monoclonal Antibody (MAB3878, Millipore, Temecula, CA, USA) in 10% milk at 4°C overnight. After incubation with goat anti-rabbit secondary antibody (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA), Western blot images were captured and analyzed by a Chemi Doc system (Bio-Rad). An anti-CREB antibody (RB-17000P0; NeoMarkers, Fremont, CA, USA) was used as an internal control for each sample.

Statistical analysis

Values are expressed as means $\pm$ SEM and differences between C/C and HF/C were evaluated using least significant differences *pos hoc* tests after one-way analysis of variance. Level of significance was set at $P < 0.05$. All analyses were conducted using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA).

Results

Body weight, food intake and serum analysis

The HF fed dams were slightly heavier than controls during the gestational period at the end of gestation (369.6 $\pm$ 10.3 g versus 355.4 $\pm$ 11.5 g), but the difference was not statistically significant (Figure 1a). The offspring were weighed within 24 h after birth and weekly thereafter. The body weight of HF/C was higher than that of C/C from week 2 to week 6, while the body weight of both groups showed no significant difference at birth and from weeks 7 to 12 (Figure 1b). The HF/C offspring had significantly higher serum glucose ($P < 0.05$) as well as ln (HOMA-IR) ($P < 0.05$) when compared with C/C. There were no significant differences in serum insulin and lipid profiles between C/C and HF/C offspring (Table 2).

Muscular histological analysis and physiological observations

The muscle fiber structure tested by using H&E staining appeared normal and did not differ qualitatively between the HF/C and C/C pups (Figure 2a). However, image quantification results showed that the mean area of muscle fiber was smaller in HF/C compared with C/C muscle ($P < 0.05$, Figure 2c). The wet weight of bilateral gastrocnemius (trimmed of fat and connective tissues) in C/C was heavier than the HF/C pups ($P < 0.05$,

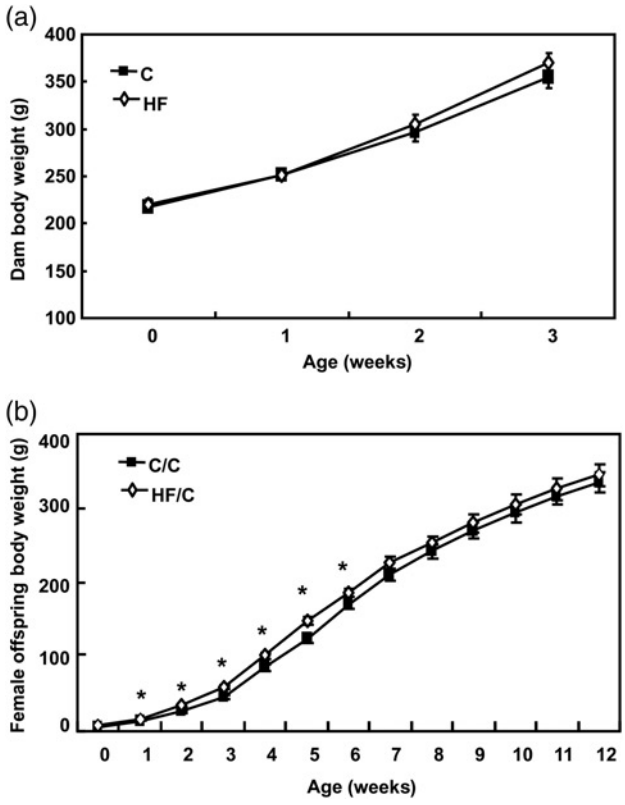

Figure 1 (a) Body weight curve of dams that were either exposed to a control (C) or high-fat (HF) diet diet during gestation and lactation ($n = 6$). (b) Body weight curve of female offspring of C/C and HF/C groups ($n = 10$). The values represent the mean ± SEM, $^*P < 0.05$ when compared with C or C/C groups

Table 2 Serum physiological outcomes in female offspring at the age of week 12

	C/C	HF/C
Serum glucose (mmol/L)	4.97 ± 0.28	5.58 ± 0.26*
Serum insulin (pmol/L)	200.36 ± 16.85	209.76 ± 27.43
ln (HOMA-IR)	1.84 ± 0.09	2.00 ± 0.11*
Serum TG (mmol/L)	0.98 ± 0.17	1.03 ± 0.20
Serum TC (mmol/L)	1.76 ± 0.29	1.63 ± 0.22

Values are mean ± SEM, $n = 10$. $^*P < 0.05$ to C/C versus HF/C
Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) = serum glucose (mmol/L) × serum insulin (pmol/L)/(22.5 × 6.965), and then the natural logarithm of HOMA-IR was taken to become normal distribution

Figure 2e). Differences between lipid droplets were observed in Oil Red O staining between the HF/C and C/C pups. The red droplets stained in the HF/C pups were more than those observed in the C/C pups in all samples examined (Figure 2b). The quantitative determination revealed that the intramuscular triglyceride content was about 1.8-fold higher in the HF/C than in the C/C group ($P < 0.05$, Figure 2d). Furthermore, the muscle glycogen concentration in the HF/C was lower than that of C/C pups ($P < 0.05$, Figure 2f).

Wnt gene mRNA expression in skeletal muscle

The mRNA expressions of the Wnt signaling family, including Wnt1, Wnt3a and Wnt5a, was examined. At the age of three weeks, there was no difference in Wnt1 and Wnt5a gene mRNA expression between C/C and HF/C offspring, whereas Wnt3a expression was higher in C/C than in HF/C (Figure 3a). At the age of 12 weeks, the mRNA expression of Wnt1 decreased 1.9-fold ($P < 0.05$) in HF/C female offspring compared with that of C/C, and the mRNA expression of Wnt3a decreased 2.0-fold ($P < 0.05$) in HF/C female offspring when compared with C/C, while that of Wnt5a did not change (Figure 3b).

Nuclear β-catenin and MyoD protein concentrations in skeletal muscle

Nuclear β-catenin accumulation is the marker of the activated canonical Wnt signaling pathway and results in the activation of target genes. To detect the nuclear β-catenin protein concentration in female offspring skeletal muscle, nuclear protein samples were subjected to Western blot analysis. Nuclear β-catenin protein was significantly lower in the skeletal muscle of HF/C female offspring when compared with C/C at weeks 3 and 12 (Figure 4a; $P < 0.05$), a result that is consistent with the trend observed in the mRNA expression of Wnt genes. The Wnt/β-catenin signaling pathway has been reported to activate MyoD in stem cells.[20] In the current study, the MyoD protein concentration was down-regulated by 2.0-fold in HF/C female offspring when compared with C/C at week 3 ($P < 0.05$). Although the MyoD protein concentration in the HF/C was also lower than the C/C group at week 12, the difference was not significant ($P = 0.07$) (Figure 4b). This result indicates that altered Wnt/β-catenin signaling decreased the MyoD protein concentration in the skeletal muscle of female offspring.

Discussion

It is well recognized that a maternal HF diet has a long-term influence on fetal and postnatal development.[21–23] Our experiment indicates that prenatal and early postnatal exposure to a high-saturated-fat diet increased fasting serum glucose and HOMA-IR significantly in female adult offspring. HF/C rat offspring also exhibited an increased intramuscular fat accumulation and decreased muscle glycogen concentration. Excessive intramuscular fat accumulation is considered to disrupt normal muscle insulin sensitivity and therefore constitutes a reliable marker of whole-body insulin resistance,[24] while muscle glycogen content is negatively correlated with fasting hyperglycemia and insulin resistance, and so can be viewed as a marker of the development of glucose intolerance.[25] Summing up the serum and muscle physiological observations, our rat model demonstrated detectable signs of increased propensity for insulin resistance after prenatal and early postnatal exposure to a high-saturated-fat diet.

Skeletal muscle is an essential site of insulin resistance in the context of metabolic disease, such as type 2 diabetes and obesity.[26–28] The early life stage is crucial for skeletal muscle development. Bayol *et al.*[29] reported adult offspring from mothers fed Westernized 'junk food' (rich in fat, sugar

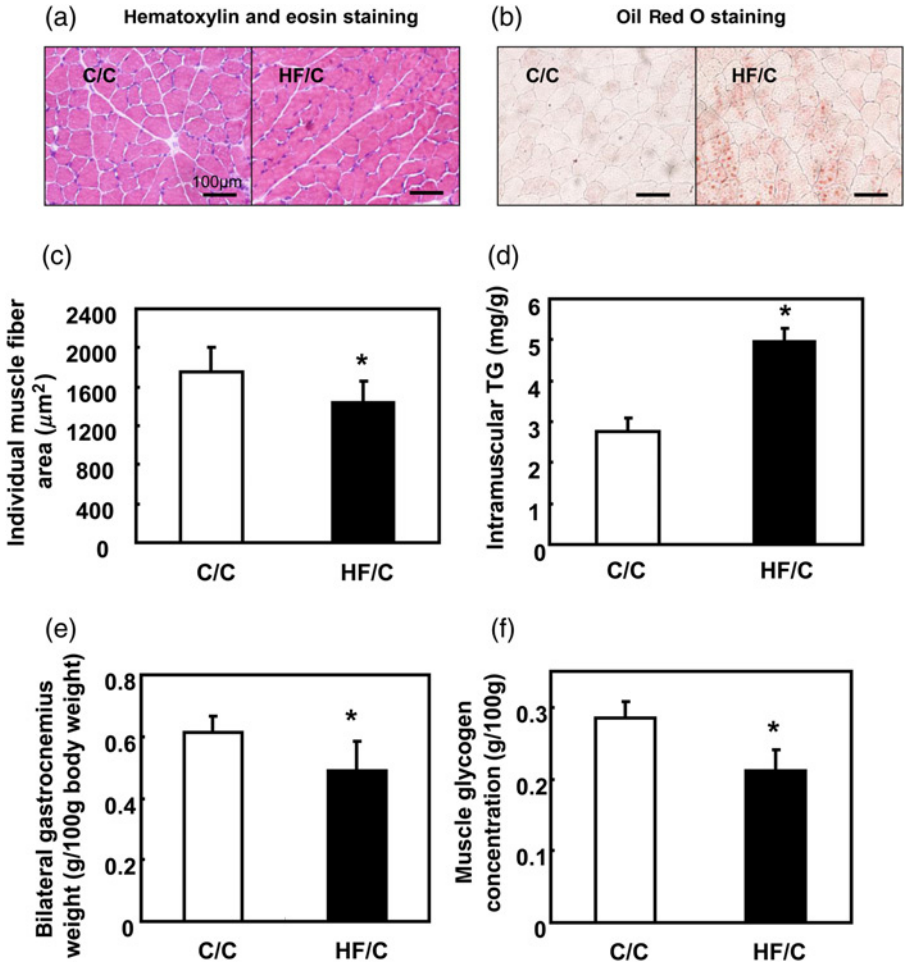

Figure 2 Muscular physiological observations in female offspring at age 12 weeks. Histological assays including hematoxylin and eosin (a) and Oil Red O staining (b) of gastrocnemius of female offspring (×200 magnification). Individual muscle fiber area (c), intramuscular triglyceride concentration (d), bilateral gastrocnemius wet weight (e) and muscle glycogen concentration (f). Values are means ± SEM, $n = 10$. $^{*}P < 0.05$. (A color version of this figure is available in the online journal)

and salt) in pregnancy and lactation displayed reduced muscle force regardless of the post-weaning diet. The present study showed decreased wet weight of gastrocnemius and smaller muscle fiber area in HF/C pups, which indicated prenatal and early postnatal exposure to a high-saturated-fat diet impaired skeletal muscle development. Stannard *et al.* reported that poor fetal skeletal muscle development impairs glucose and fatty acid metabolism by skeletal muscle in response to insulin stimulation, and thus predisposes offspring for developing diabetes and obesity in adulthood.

By conveying information from the cell surface to the nucleus, the Wnt signaling pathway regulates embryonic development and influences cell fate determination. The Wnt family, including wnt1, wnt3a and wnt5a, act by binding to Frizzled proteins on target cells, which, in turn, induce the signaling cascade and lead to an accumulation of β-catenin in the nucleus.[30,31] Acting in a complex with members of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors, β-catenin activates its target gene transcription.[32,33] In the current study, we observed that prenatal and early postnatal exposure to a high-saturated-fat diet suppressed the canonical Wnt/β-catenin signaling pathway in female offspring, including the mRNA expression of Wnt1 and Wnt3a and nuclear accumulation of β-catenin protein.

There are studies confirming that Wnt/β-catenin signaling plays vital roles in muscle development, during both the embryogenic specification of muscle and adult muscle regeneration.[34,35] The activation of the Wnt signaling pathway leads to the transformation of nonmyogenic cells into the myogenic lineage.[36,37] MyoD-mediated transactivation was inhibited in muscle cells when β-catenin was deficient or the interaction between MyoD and β-catenin was disrupted, demonstrating that β-catenin directly interacts with MyoD and enhances its transcriptional activity to induce muscle differentiation.[38] In this study, consistent with the reduced protein concentrations of nuclear β-catenin in the skeletal muscle of HF/C offspring, the protein concentration of MyoD, a myogenic marker,[39,40] was also decreased in HF/C offspring, suggesting that MyoD is likely down-regulated by the Wnt/β-catenin signaling pathway.

Skeletal muscle usually is made up of a mixture of fast glycolytic and slow oxidative twitch muscle fibers, and the ratio of fast twitch to slow twitch muscle determines

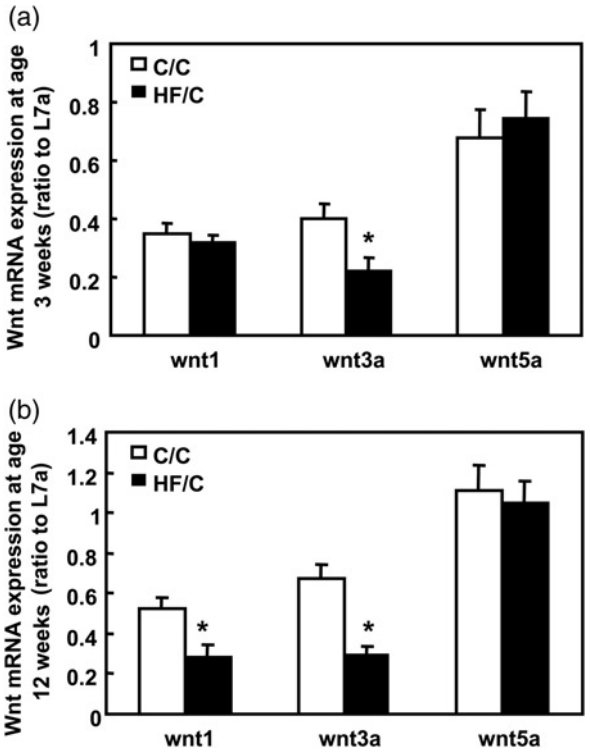

Figure 3 Wnt1, Wnt3a and Wnt5a mRNA expression levels in female offspring skeletal muscle at the age of week 3 (a). Wnt1, Wnt3a and Wnt5a mRNA expression levels in female offspring skeletal muscle at the age of week 12 (b). Data are presented in each unit as relative mRNA abundance normalized to rL7a transcript abundance. The bar values represent the mean ± SEM ($n = 10$). *Indicates comparison of Wnt mRNA levels in C/C and HF/C groups, $P < 0.05$

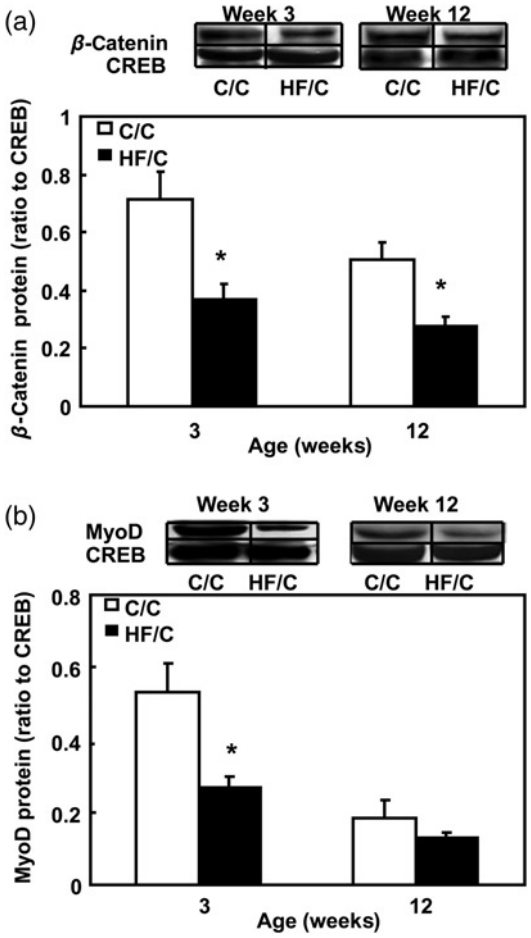

Figure 4 β-Catenin (a) and MyoD (b) protein concentrations in female offspring skeletal muscle. The bars represent the average level of protein in C/C and HF/C groups. The intensity of the β-catenin and MyoD bands were normalized to that of the CREB band. The values represent the mean ± SEM, $n = 10$, *$P < 0.05$ when compared with C/C group

the metabolic properties and the function of individual muscle.[41] It was reported that MyoD transcripts correlate with the proportions of fast glycolytic and slow oxidative muscle fibers, and MyoD is prevalent in fast twitch muscle.[42] In the present study, gastrocnemius muscle, containing both glycolytic and oxidative muscle, was chosen to determine the MyoD protein concentration. In future studies, other types of muscle, such as slow oxidative twitch muscle, should be examined to understand better the influence of maternal HF diet on the development of different types of muscle.

In summation, the current study showed that the female offspring of high-saturated-fat-diet-fed dams presented a tendency of development of insulin resistance as well as lesion of muscle development at age 12 weeks. In addition, we observed the repression of Wnt/β-catenin signaling and myogenic genes by prenatal and early postnatal exposure to a high-saturated-fat diet. Taken together, we suggest that prenatal and early postnatal exposure to high-saturated fat suppresses the development of skeletal muscle and myogenic genes via Wnt/β-catenin signaling in female offspring, and this inappropriate muscle development during early life could potentially contribute to the predisposition of offspring to develop a metabolic-syndrome-like phenotype in adulthood.

Author contributions: WC and K-FY designed the experiment. K-FY conducted the animal experiments and analysed the data; X-HS assisted with laboratory analysis and data collection; K-FY drafted the manuscript; and WC gave important advice to paper writing. All authors have approved the manuscript and agreed with submission to *Experimental Biology and Medicine*.

ACKNOWLEDGEMENTS

The authors appreciate the adivce provided by Dr Yuan-Xiang Pan (Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign) in designing the experiment.

The present study was supported by the National Natural Science Foundation of China (No. 30772270, 30972427) and Program for Innovative Research Team of Shanghai Municipal Education Commission.

REFERENCES

1 Kimokoti RW, Millen BE. Diet, the global obesity epidemic, and prevention. *J Am Diet Assoc* 2011;**111**:1137–40

2 Lichtenstein AH, Kennedy E, Barrier P, Danford D, Ernst ND, Grundy SM, Leveille GA, Van Horn L, Williams CL, Booth SL. Dietary fat consumption and health. *Nutr Rev* 1998;**56**:S3–19

3 Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care* 2011;**34**:1249–57

4 Zhai F, Wang H, Du S, He Y, Wang Z, Ge K, Popkin BM. Prospective study on nutrition transition in China. *Nutr Rev* 2009;**67**(Suppl. 1): S56–61

5 Martin-Gronert MS, Ozanne SE. Mechanisms linking suboptimal early nutrition and increased risk of type 2 diabetes and obesity. *J Nutr* 2010;**140**:662–6

6 Chechi K, Cheema SK. Maternal diet rich in saturated fats has deleterious effects on plasma lipids of mice. *Exp Clin Cardiol* 2006;**11**:129–35

7 Godfrey KM, Barker DJ. Fetal programming and adult health. *Public Health Nutr* 2001;**4**:611–24

8 Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992;**35**:595–601

9 Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* 2001;**60**:5–20

10 Bayol SA, Farrington SJ, Stickland NC. A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring. *Br J Nutr* 2007;**98**:843–51

11 Buckley AJ, Keseru B, Briody J, Thompson M, Ozanne SE, Thompson CH. Altered body composition and metabolism in the male offspring of high fat-fed rats. *Metabolism* 2005;**54**:500–7

12 Chechi K, Herzberg GR, Cheema SK. Maternal dietary fat intake during gestation and lactation alters tissue fatty acid composition in the adult offspring of C57Bl/6 mice. *Prostaglandins Leukot Essent Fatty Acids* 2010;**83**:97–104

13 Rising R, Lifshitz F. Lower energy expenditures in infants from obese biological mothers. *Nutr J* 2008;**7**:15

14 DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 1981;**30**:1000–7

15 Jin T. The WNT signalling pathway and diabetes mellitus. *Diabetologia* 2008;**51**:1771–80

16 Schinner S, Willenberg HS, Schott M, Scherbaum WA. Pathophysiological aspects of Wnt-signaling in endocrine disease. *Eur J Endocrinol* 2009;**160**:731–7

17 Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 1993;**123**:1939–51

18 Guo Z, Mishra P, Macura S. Sampling the intramyocellular triglycerides from skeletal muscle. *J Lipid Res* 2001;**42**:1041–8

19 Kim CH, Kim MS, Youn JY, Park HS, Song HS, Song KH, Park JY, Lee KU. Lipolysis in skeletal muscle is decreased in high-fat-fed rats. *Metabolism* 2003;**52**:1586–92

20 Ridgeway AG, Petropoulos H, Wilton S, Skerjanc IS. Wnt signaling regulates the function of MyoD and myogenin. *J Biol Chem* 2000;**275**:32398–405

21 Ferezou-Viala J, Roy AF, Serougne C, Gripois D, Parquet M, Bailleux V, Gertler A, Delplanque B, Djiane J, Riottot M, Taouis M. Long-term consequences of maternal high-fat feeding on hypothalamic leptin sensitivity and diet-induced obesity in the offspring. *Am J Physiol Regul Integr Comp Physiol* 2007;**293**:R1056–62

22 Chang GQ, Gaysinskaya V, Karatayev O, Leibowitz SF. Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity. *J Neurosci* 2008;**28**:12107–19

23 Elahi MM, Cagampang FR, Mukhtar D, Anthony FW, Ohri SK, Hanson MA. Long-term maternal high-fat feeding from weaning through pregnancy and lactation predisposes offspring to hypertension, raised plasma lipids and fatty liver in mice. *Br J Nutr* 2009;**102**:514–9

24 Hegarty BD, Furler SM, Ye J, Cooney GJ, Kraegen EW. The role of intramuscular lipid in insulin resistance. *Acta Physiol Scand* 2003;**178**:373–83

25 He J, Kelley DE. Muscle glycogen content in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab* 2004;**287**:E1002–7

26 Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. *Science* 2005;**307**:384–7

27 Ozanne SE, Jensen CB, Tingey KJ, Storgaard H, Madsbad S, Vaag AA. Low birthweight is associated with specific changes in muscle insulin-signalling protein expression. *Diabetologia* 2005;**48**:547–52

28 Bouzakri K, Koistinen HA, Zierath JR. Molecular mechanisms of skeletal muscle insulin resistance in type 2 diabetes. *Curr Diabetes Rev* 2005;**1**:167–74

29 Bayol SA, Macharia R, Farrington SJ, Simbi BH, Stickland NC. Evidence that a maternal "junk food" diet during pregnancy and lactation can reduce muscle force in offspring. *Eur J Nutr* 2009;**48**:62–5

30 Peifer M, Polakis P. Wnt signaling in oncogenesis and embryogenesis – a look outside the nucleus. *Science* 2000;**287**:1606–9

31 Willert K, Jones KA. Wnt signaling: is the party in the nucleus? *Genes Dev* 2006;**20**:1394–404

32 Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. *J Cell Sci* 2003;**116**:1175–86

33 Ying Y, Tao Q. Epigenetic disruption of the WNT/beta-catenin signaling pathway in human cancers. *Epigenetics* 2009;**4**:307–12

34 Armstrong DD, Esser KA. Wnt/beta-catenin signaling activates growth-control genes during overload-induced skeletal muscle hypertrophy. *Am J Physiol Cell Physiol* 2005;**289**:C853–9

35 Petropoulos H, Skerjanc IS. Beta-catenin is essential and sufficient for skeletal myogenesis in P19 cells. *J Biol Chem* 2002;**277**:15393–9

36 Polesskaya A, Seale P, Rudnicki MA. Wnt signaling induces the myogenic specification of resident CD45+ adult stem cells during muscle regeneration. *Cell* 2003;**113**:841–52

37 Otto A, Schmidt C, Luke G, Allen S, Valasek P, Muntoni F, Lawrence-Watt D, Patel K. Canonical Wnt signalling induces satellite-cell proliferation during adult skeletal muscle regeneration. *J Cell Sci* 2008;**121**:2939–50

38 Kim CH, Neiswender H, Baik EJ, Xiong WC, Mei L. Beta-catenin interacts with MyoD and regulates its transcription activity. *Mol Cell Biol* 2008;**28**:2941–51

39 Megeney LA, Kablar B, Garrett K, Anderson JE, Rudnicki MA. MyoD is required for myogenic stem cell function in adult skeletal muscle. *Genes Dev* 1996;**10**:1173–83

40 Tanaka Y, Yamaguchi A, Fujikawa T, Sakuma K, Morita I, Ishii K. Expression of mRNA for specific fibroblast growth factors associates with that of the myogenic markers MyoD and proliferating cell nuclear antigen in regenerating and overloaded rat plantaris muscle. *Acta Physiol (Oxf)* 2008;**194**:149–59

41 Spangenburg EE, Booth FW. Molecular regulation of individual skeletal muscle fibre types. *Acta Physiol Scand* 2003;**178**:413–24

42 Maves L, Waskiewicz AJ, Paul B, Cao Y, Tyler A, Moens CB, Tapscott SJ. Pbx homeodomain proteins direct MyoD activity to promote fast-muscle differentiation. *Development* 2007;**134**:3371–82

(Received November 24, 2011, Accepted April 9, 2012)