Eur J Nutr (2013) 52:1181–1189
DOI 10.1007/s00394-012-0428-z

ORIGINAL CONTRIBUTION

Differential effects of high-fat-diet rich in lard oil or soybean oil on osteopontin expression and inflammation of adipose tissue in diet-induced obese rats

Xiaoke Wang · Mengjie Cheng · Min Zhao · Aiguo Ge · Fangfang Guo · Min Zhang · Yanhong Yang · Liegang Liu · Nianhong Yang

Received: 11 January 2012 / Accepted: 16 July 2012 / Published online: 31 July 2012

Abstract
Purpose To examine the effect of different dietary fat types on osteopontin (OPN) expressions and inflammation of adipose tissues in diet-induced obese rats.
Methods Male Sprague–Dawley rats were randomly assigned to one control group fed standard diet (LF, $n = 10$) and two high-fat diet groups fed isoenergy diet rich in lard or soybean oil (HL or HS, $n = 45$ each). Diet-induced obese rats in HL and HS group were then subdivided into two groups either continuously fed high-fat diet or switched to low-fat diet for 8 more weeks. Fasting serum glucose, insulin, and OPN concentrations were assayed and QUICKI was calculated; the expression of OPN, IL-6, IL-10, TNF-α, NF-κB, and F4/80 in adipose tissue was determined.
Results Both high-fat diets lead to comparable development of obesity characterized by insulin resistance and adipose tissue inflammation. Obese rats continuously fed high-fat diet rich in lard oil exhibited the highest fasting serum insulin level and adipose tissue OPN, F4/80, TNF-α, and NF-κB expression level. In both high-fat diet groups, switching to low-fat diet resulted in less intra-abdominal fat mass, decreased expression of F4/80, TNF-α, and NF-κB, while decreased OPN expression was only observed in lard oil fed rats after switching to low-fat diet.
Conclusions Reducing diet fat or replacing lard oil with soybean oil in high-fat diet alleviates obesity-related inflammation and insulin resistance by attenuating the upregulation of OPN and macrophage infiltration into adipose tissue induced by high-fat diet.

Keywords Diet-induced obese · Inflammation · Insulin resistance · Osteopontin · High-fat diet · Soybean oil · Lard oil

Abbreviations

GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HL	High-fat diet rich in lard
HS	High-fat diet rich in soybean oil
LF	Low-fat standard chow
MUFA	Monounsaturated fatty acid
NF-κB	Nuclear factor kappa B
OPN	Osteopontin
PPARγ	Peroxisome proliferator-activated receptor gamma
PUFA	Polyunsaturated fatty acids
QUICKI	Quantitative insulin sensitivity check index
TNF-α	Tumor necrosis factor-α
IL-6	Interleukin-6
IL-10	Interleukin-10

Introduction

Obesity is associated with chronic low-grade inflammation characterized by macrophage infiltration into adipose tissue, elevated inflammatory factors, and insulin resistance [1]. Recent studies indicated that adipose tissue macrophages accumulation during diet-induced obesity not only is an important source of adipose tissue inflammation but

X. Wang · M. Cheng · M. Zhao · A. Ge · F. Guo · M. Zhang · Y. Yang · L. Liu · N. Yang (✉)
Department of Nutrition and Food Hygiene, MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Rd, Wuhan 430030, Hubei Province, People's Republic of China
e-mail: zynh@mails.tjmu.edu.cn

also alters insulin sensitivity in adipocytes, probably through activation of inflammation signaling pathway, such as NF-κB pathway in adipose tissue [2–4]. The macrophages recruited to adipose tissue during the development of obesity belong to the "pro-inflammatory" M1-phenotype, which are highly activated, sensitive to lipopolysaccharide and free fatty acids, express F4/80 and CD11c markers, and produce a wide array of pro-inflammatory cytokines such as TNF-α and IL-6 [2, 5, 6].

Osteopontin (OPN), also named secreted phosphoprotein-1 and sialoprotein-1, is a multifunctional phosphorylated glycoprotein expressed in a wide range of cells including activated macrophages and T-cells [7–11]. Characterized as a macrophage-chemotactic stimulus and proinflammatory cytokine, OPN plays a role in various inflammatory disorders, such as rheumatoid arthritis [12], atherosclerosis [13], and hepatic inflammation [14]. Recent research shows that OPN expression is extensively upregulated in adipose tissue upon human and murine obesity and functionally involved in the pathogenesis of obesity-induced adipose inflammation and associated insulin resistance [15, 16]. OPN knockout mice fed with high-fat diet were protected from the skeletal muscle, liver, and adipose tissue insulin resistance which was developed in wild-type controls [17, 18]. Interference with OPN action by a neutralizing antibody for 5 days also significantly reduced obesity-related insulin resistance and inflammation [19].

Given the proposed key role of OPN in metabolic deterioration resulting from chronic obesity, no study has yet addressed the question whether the amount and types of dietary fat influence OPN expression and inflammation in adipose tissues of dietary-induced obese animals. In the present study, by feeding rats with isoenergy high-fat diet rich in either lard (HL) or soybean oil (HS) to induce obese, then switching to low-fat chow, we examined the effect of different dietary fat type on osteopontin (OPN) expressions and inflammation of adipose tissues in diet-induced obese rats.

Materials and methods

Animals and diets

Male Sprague–Dawley rats at 5–6 weeks of age and weighing 120–130 g ($n = 100$, purchased from Shanghai Sippr-VK lab animal Co. Ltd.) were housed individually in cages in pathogen-free, temperature- and humidity-controlled (17–27 °C; 40–60 % relative humidity) rooms with a 12/12 h light/dark cycle. All rats had ad libitum access to their respective food and water throughout the study. Food consumption and body weight were measured daily and weekly. After a week of standard rodent chow feeding to allow them to adapt to the new environment, blood sample was taken from the tail vein to determine the serum OPN concentrations. The rats were then randomly divided into 3 groups and fed with either low-fat standard chow (LF, $n = 10$) or high-fat diets rich in lard (HL, $n = 45$) or soybean oil (HS, $n = 45$). The standard chow was purchased from Laboratory Animal Centre of Tongji Medical College, Huazhong University of Science and Technology, China; the high-fat diets were prepared by adding additional fats and other components. The detailed compositions were given in Table 1. Except added fat, the other compositions of HL and HS were all the same. At the end of the 10th week, rats fed with HL or HS diet with body weights more than $\bar{x} \pm 2$ s of LF group were classified as diet-induced obese and selected for further study. The obese rats from HL group and HS group were then randomly subdivided into two groups of similar mean body weight. One group was continuously fed with high-fat diet (HL/HL and HS/HS), and the other group was switched to LF (HL/LF and HS/LF) for another 8 weeks. All rats were killed after 12 h fasting, and trunk blood was collected and serum samples were stored at −80 °C until assay. White adipose tissues from epididymal and perirenal locations were dissected, weighed, and immediately snap-frozen in liquid nitrogen, and then stored at −80 °C until analysis. Body fat index was calculated as [(perirenal pad + epididymal pad)/final body weight] × 100 %. All experiments were conducted in accordance with national and institutional guidelines for the protection of human subjects

Table 1 Composition of the study diets (g/100 g)

	Low-fat chow diet (LF)	High-fat diet rich in lard oil (HL)	High-fat diet rich in soybean oil (HS)
Standard chow (g)	100	60	60
Lard oil (g)	–	15	–
Soybean oil (g)	–	0	15
Egg yolk power (g)	–	13	13
Casein (g)	–	10	10
Sugar (g)	–	2	2
Protein (kJ %)	22.0	20.0	20.0
Carbohydrate (kJ %)	64.4	30.2	30.2
Starch	64.4	28.5	28.5
Sugar	–	1.7	1.7
Fat (kJ %)	13.7	40.8	40.8
Saturated	3.4	20.9	7.4
Monounsaturated	3.2	21.9	12.0
Polyunsaturated (n6)	7.9	5.0	19.2
Polyunsaturated (n3)	–	–	1.8
Density (kJ/g)	13.8	19.3	19.3

and animal welfare. The protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Tongji Medical College.

Analysis of blood samples

The serum glucose concentrations were determined by the glucose oxidase method using a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China), and the serum insulin concentrations were measured by radioimmunoassay (Chemclin Biotechnology Corporation Limited, Beijing, China). Quantitative insulin sensitivity check index (QUICKI) was calculated according to the following equation: 1/[Log (fasting insulin, μU/ml) + Log (fasting glucose, mg/dl)] [20, 21]. The serum OPN concentration was measured by enzyme-linked immunosorbent assay (R&D Systems, CA, USA), and fasting serum cholesterol was measured by an enzymatic colorimetric method (Jiancheng Biotechnology Corporation Limited, Nanjing, China).

RNA isolation and real-time quantitative PCR analysis

Total RNA was isolated from perirenal adipose tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. Using the RT system (Takara Bio, Dalian, China), cDNA was synthesized from 2 μg mRNA at 37 °C for 15 min followed by 85 °C for 5 s. Real-time quantitative PCR was performed using the ABI 7900HT real-time thermocycler (Applied Biosystems, Forster, CA) and SYBR Premix Ex TaqTM (Takara Bio, Dalian, China). The PCR thermal cycle conditions were 95 °C for 2 min followed by 40 cycles of 90 °C for 5 s, 60 °C for 30 s, and 72 °C for 30 s. Each sample was run in triplicate, negative control samples and reactions mixed without cDNA templates were run in parallel. GAPDH was used as an endogenous control to indicate relative quantification of gene expression from each sample. The results of real-time PCR were analyzed with the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers for the genes in this study were as follows. OPN: forward 5′-AGCACACAAGCA GACGTTTT-3′, reverse 5′-TGGGATGACCTTGATAG CCT-3′; F4/80: forward 5′-AAG GAT ACG AGG TTG CTG AC-3′, reverse 5′-CCT GAA GGC TGT TGA TAG TG-3′; NF-κB: forward 5′-ATCTGTTTCCCCTC ATCTTTC-3′, reverse 5′-GTGCGTCTTAGTGGTATCT GTG-3′; TNF-α: forward 5′-GGAAAGCATGATCCGAG A TG-3′, a reverse 5′-CAGTAGACAGAAGAGCGTG GTG-3′; IL-6: forward 5′-TTGCCTTCTTGGGACTGAT G-3′, reverse 5′-ACTGGTCTGTTGTGGGTGGT-3′; IL-10: forward 5′-AGGGTTACTTGGGTTGC-3′, reverse 5′-ATG CTCCTTGATTTCTGG-3′; GAPDH: forward 5′-GCAA GTTCAACGGCACAG-3′, reverse 5′-GCCAGTAGACT CCACGACAT-3′.

Electrophoresis and Western blotting

Adipose tissue was homogenized in ice-cold lysis buffer containing 50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 % Nonidet P-40, 1 % sodium deoxycholate, 1 % SDS, 0.1 mM dithiothreitol, 0.05 mM phenylmethyl sulfonylfluoride, 10 mM NaF, 0.5 mM Na_3VO_4, and protease inhibitor cocktail (Amresco, Solon, OH, USA) and then left at 4 °C for 2 h. The supernatant was then collected by centrifugation (10,000*g* for 15 min at 4 °C) and protein content was determined by DC protein assay (Bio-Rad, Hercules, CA, USA). Equal amount of protein was mixed with SDS sample buffer and incubated for 5 min at 98 °C before loading. Protein samples (40 μg) were resolved on 10 % SDS–polyacrylamide gels, separated and electroblotted onto Hybond-P polyvinylidene difluoride transfer membranes (Amersham, CA, USA). The membranes were blocked overnight at 4 °C with TBS-T (50 mM Tris–HCl, pH7.5, 0.5 % Tween 20, 150 mM NaCl) containing 5 % nonfat milk. Dilutions of specific primary and horseradish peroxidase-conjugated secondary antibodies (Pierce, USA, 1:10,000) were made in 0.05 % Tween 20 in PBS, pH 7.4. Incubation of blots was performed for 2 h at room temperature with a specific primary antibody followed by secondary antibody. Chemiluminescence was generated by BM chemiluminescence substrate (Roche, Mannheim, Germany) and quantified from scanned autoradiograms using NIH Image. The specific primary antibodies used were OPN monoclonal antibody (sc-21742; Santa Cruz, CA, USA, 1:400) and β-actin monoclonal antibody (8H10D10; Cell Signaling, CA, USA, 1:1,000).

Statistical analysis

Data were expressed as mean ± SEM. One-way ANOVA was used to compare differences among multiple groups, and Student–Newman–Keuls post hoc test was used to determine differences between individual groups. Two-way ANOVA was used to analyze the effects of different high-fat diets, switching from a high-fat diet to low-fat chow and the interaction between. All statistical analyses were performed using SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA), and a difference was considered significant when $P < 0.05$.

Results

Food intake, body weight, and adiposity

At the end of the 10th week, 18 of HL group (40 %) and 17 of HS group (38 %) rats were designated as diet-induced obese according to the body weight as compared with that

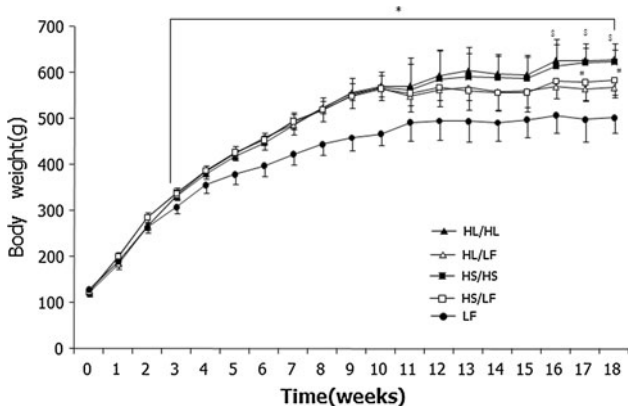

Fig. 1 Differential effects of high-fat diets rich in lard or soybean oil on body weight of male Sprague–Dawley rats. Body weights were measured weekly. Data were presented as mean ± SEM. *$P < 0.05$ versus LF group, #$P < 0.05$ versus corresponding group received respective continuous high-fat diet, $$P < 0.05$ versus corresponding group received HS

of LF group. Obese rats showed significant increase of body weight than LF rats starting from the 3rd week and persisting throughout the entire study (Fig. 1). No significant difference in body weight was found between obese rats fed with HL and HS. Compared with HL/HL and HS/HS group rats which received continuous high-fat diets, both HL/LF and HS/LF group rats showed significant reduction in body weight after switching to low-fat diet for 8 weeks ($P < 0.05$). During the first 10 weeks, both the cumulative total energy intake and the energy intake from fat in HL and HS group obese rats were higher than those in LF group rats. No significant difference was observed between HL and HS group. During the following 8 weeks, no significant difference of cumulative energy intake was observed among all 5 groups, even though the energy intake from fat was lower in both HL/LF and HS/LF groups than that in HL/HL and HS/HS groups which received continuous high-fat diet (Table 2).

Intra-abdominal fat pads

The amount of intra-abdominal fat pads was presented here as total perirenal and epididymal fat masses from both sides of the body. The HL/HL group had the largest amount of perirenal fat, epididymal fat, and the highest body fat index among the 5 groups. The HS/HS group exhibited significantly less body fat index as compared to HL/HL although they showed no significant difference in initial body weight, weight gain, and energy intake throughout the experiments. Both HL/LF and HS/LF had reduced body fat index when compared to their respective counterparts which received continuous high-fat diet (Table 3).

Serum glucose, insulin, total cholesterol, and OPN concentrations

As shown in Table 3, no significant differences were shown in serum glucose, insulin, total cholesterol, and OPN levels among all groups at the beginning of the experiment. After 18 weeks of respective feeding, HL/HL group has significantly higher serum insulin concentrations and lower QUICKI than all other groups ($P < 0.05$); both HL/LF and HS/LF groups have decreased serum insulin concentrations and higher QUICKI than their respective counterparts maintained on high-fat diet. No significant differences were observed in fasting serum total cholesterol and OPN concentrations among all 5 groups either at the beginning or at the end of the experiment.

OPN mRNA and protein expressions in adipose tissue

Both mRNA and protein level of OPN were significantly higher in the HL/HL rats compared to all other groups while switching to low-fat chow reversed this increase in HL/LF rats. No significant increase of OPN expression was found in HS/HS rats (Fig. 2).

Table 2 Accumulated energy intake and energy from fat of each group in different periods

	LF	HL/HL	HL/LF	HS/HS	HS/LF
Number of rats	10	8	8	8	8
0–10th week					
Total energy intake	27,633 ± 1,381	30,527 ± 2,037*	30,845 ± 1,685*	31,509 ± 1,674*	30,804 ± 2,534*
Energy from fat	3,786 ± 189	12,456 ± 831*	12,585 ± 687*	12,856 ± 683*	12,568 ± 1.34*
11–18th week					
Total energy intake	17,521 ± 1,960	18,911 ± 2,067	17,403 ± 2,033	19,163 ± 2,308	18,336 ± 2,230
Energy from fat	2,400 ± 269	7,716 ± 843*	2,384 ± 278#	7,819 ± 942*	2,512 ± 306#

Data are presented as mean ± SEM

* $P < 0.05$ versus LF group

# $P < 0.05$ versus corresponding group received respective continuous high-fat diet

Table 3 Visceral fat pad, fasting serum glucose, insulin, total cholesterol, OPN and QUICKI of rats with different diet regime

	LF	HL/HL	HL/LF	HS/HS	HS/LF
Number of rats	10	8	8	8	8
Perirenal fat (g)	8.45 ± 2.55	23.39 ± 4.97*,$	11.98 ± 2.37#	15.75 ± 4.37*	9.95 ± 3.28#
Epididymal fat (g)	9.59 ± 3.17	20.35 ± 2.06*,$	12.09 ± 1.86#	15.98 ± 4.87*	12.55 ± 2.30#
Perirenal + Epididymal fat (g)	18.04 ± 6.00	43.74 ± 4.79*,$	24.07 ± 3.58*,#	29.98 ± 7.45*	22.14 ± 5.28#
Body fat index	3.64 ± 1.02	7.17 ± 0.56*,$	4.37 ± 0.55#	5.03 + 0.94*	3.95 + 0.68#
Glucose (mM)					
0th week	3.34 ± 0.72	3.16 ± 0.82	2.93 ± 0.34	3.02 ± 0.35	3.26 ± 0.25
18th week	5.04 ± 0.47	5.16 ± 0.44	4.33 ± 0.68	5.28 ± 0.40	4.89 ± 0.41
Insulin (pM)					
0th week	187.2 ± 18.4	200.0 ± 12.5	185.7 ± 14.2	189.9 ± 23.2	196.5 ± 16.4
18th week	194.4 ± 26.5	583.5 ± 68.5*,$	418.9 ± 32.4*,#	359.8 ± 36.2*	234.3 ± 33.4#
Total cholesterol (mM)					
0th week	1.34 ± 0.16	1.54 ± 0.18	1.47 ± 0.22	1.30 ± 0.09	1.33 ± 0.26
18th week	1.55 ± 0.47	1.94 ± 0.58	1.49 ± 0.28	1.94 ± 0.44	1.75 ± 0.68
QUICKI					
0th week	0.51 ± 0.04	0.53 ± 0.07	0.52 ± 0.03	0.53 ± 0.02	0.55 ± 0.04
18th week	0.47 ± 0.02	0.38 ± 0.02*,$	0.42 ± 0.03*,#	0.41 ± 0.02*	0.49 ± 0.03#
OPN (pg/ml)					
0th week	1,009.2 ± 62.7	1,026.2 ± 63.5	1,063.3 ± 99.5	1,062.8 ± 101.1	955.0 ± 51.1
18th week	890.1 ± 33.7	914.5 ± 48.3	1,026.89 ± 30.2	950.8 ± 69.2	865.5 ± 46.7

Rats were fed with different diet regimes for 18 weeks. Blood samples were obtained at the beginning and the end of treatment. Data are presented as mean ± SEM

* $P < 0.05$ versus LF group

# $P < 0.05$ versus corresponding group received respective continuous high-fat diet

$ $P < 0.05$ versus corresponding group received HS

Macrophage marker and inflammation-related genes in adipose tissue

To elucidate the inflammatory mechanism involved in adipose tissues, the mRNA levels of macrophage marker F4/80, pro-inflammatory cytokines IL-6, TNF-α, and anti-inflammatory cytokine IL-10 along with transcription factor NF-κB gene were examined using real-time PCR. As shown in Fig. 3, both high-fat diet (HL/HL and HS/HS) upregulated TNF-α, F4/80, and NF-κB expression significantly, while switching to low-fat chow (HL/LF and HS/LF) reversed this upregulation effect. It is of note that the induction effect of HL on NF-κB appeared to be much higher than that of HS. On the contrary, IL-10 expressions were significantly reduced in all the obese groups, and switching from high-fat diet to low-fat chow has no effect on IL-10 expressions.

Discussion

Genetic and environmental factors play important roles in the development of obesity, and diet is one of the main environmental factors that contribute to obesity and related metabolic diseases. Obese rats induced by high-energy and hyperlipidic diet represent appropriate obesity models and have been extensively used to study the physiological and molecular events in the development of obesity as they share similar global gene-expression patterns with obese humans [22–25]. The high-fat HL and HS diets used in the present study both contained 19.3 kJ/g, provided 40.8 % of energy from fat and had similar macronutrient compositions except enriched fat. As previously reported by us and others [26–28], Sprague–Dawley rats showed variable responses in food intake and weight gain when exposed to high-fat diet. However, in the current study, the two high-fat diet groups showed no significant differences in incidences of hyperphagia and obesity, indicating that it is the fat amount and energy density instead of fat type which plays a key role in overeating and development of obesity. Consistent with this observation, a number of previous studies showed no effect of fat type on the development of obesity when looking at high-fat diets based on mammal and plant fats, although some reports have described obesity resistance and less hypertrophy of visceral fat pads when employed fish oil-based diets [29–31]. In the present

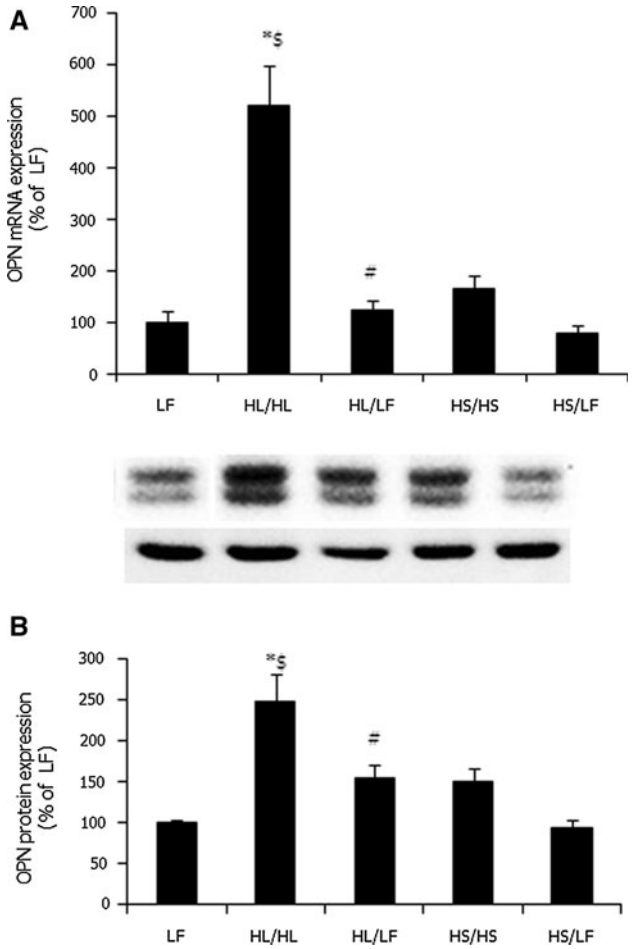

Fig. 2 Comparative effects of different diets on OPN mRNA and protein expressions in adipose tissue. **a** Relative OPN mRNA expression in adipose tissue. **b** Representative Western blot analysis of OPN and β-actin proteins in adipose tissue of rats. *Bar graph* represents OPN levels normalized to β-actin levels, which were quantified by densitometry of X-ray films. $n = 5$–6/group. Data (mean ± SEM) were given in percentage of the LF group. $^{*}P < 0.05$ versus LF group, $^{\#}P < 0.05$ versus corresponding group received respective continuous high-fat diet, $^{\$}P < 0.05$ versus corresponding group received HS

study, the amount of intra-abdominal fat was significantly lower in HS/HS group than that in HL/HL group although there is no difference in weight gain and cumulative energy consumption, suggesting that the soybean oil had a beneficial effect on lipid metabolism and fat deposition, which may be attributed to less saturated and monounsaturated fatty acid (MUFA) and more polyunsaturated fatty acids (PUFA) in soybean oil than in lard oil. Moreover, there is no cholesterol and much more vitamin E as well as flavors in soybean oil than in lard oil. In parallel with less intra-abdominal fat deposition, obese rats maintaining on HS had lower fasting blood insulin concentrations, decreased OPN, F4/80, and NF-κB expression in adipose tissue as compared to obese rats on HL [25]. Obesity is associated with chronic low-grade inflammation, and this inflammatory response appears to underlie the development of insulin resistance [3, 32–34]. The inflammatory response that emerges in the presence of obesity seems to be triggered by and to reside predominantly in adipose tissue. A lot of adipose tissue-generated cytokines have been suggested to be the main contributors of systemic inflammation in obesity and most of them are originated from macrophages [34, 35]. OPN has been found expressed predominantly in macrophages and upregulated upon obesity in adipose tissue from both obese patients and animal models of obesity [15, 16, 18, 36]. Knockout of OPN gene had no effect on the development of diet-induced obesity but did improve insulin sensitivity, decrease macrophage accumulation in adipose tissue, and protect against obesity-associated inflammation [15, 17, 18]. Importantly, OPN not only regulates macrophage migration and adipose tissue macrophages infiltration, but also is involved in adipocyte differentiation and function, and macrophages activation by inducing inflammatory signaling pathways such as IKK/IκB-α/NF-κB [37, 38]; thus, OPN may be a potential target for the treatment or prevention of obesity-related disorders. In obese rats that were switched from HL to LF diet for 8 weeks, we also observed less intra-abdominal fat mass, decreased expression of OPN, macrophage marker F4/80, inflammation-related genes IL-6, TNF-a, and NF-kB in adipose tissue of perirenal pads and improved insulin activity, which further proved the effect of dietary fat on OPN expression and the involvement of OPN in obesity-associated macrophage accumulation and inflammation in adipose tissue.

It is not clear why the high-fat diet rich in soybean oil has less induction effect on OPN expression than that rich in lard oil, but it is probably attributed to the PUFA rich in soybean oil. PUFA are known to be potent activators of PPARγ. It has been reported that OPN production was significantly reduced in response to the ligand-activated PPARγ, and the activation of PPARγ signaling in macrophages was necessary for the alternative activation of macrophages [39, 40]. PUFA could also increase lipid oxidation in white adipose tissue [41] and suppress lipogenesis [42], to a certain extent, decrease endoplasmic reticulum stress state and oxidative stress level. Endoplasmic reticulum might be a key site for the sensing of metabolic stress. High-fat diet provided lots of energy and lipids that challenges endoplasmic reticulum function and increases endoplasmic reticulum stress in adipose tissue [43], whereas endoplasmic reticulum stress is a major source of reactive oxygen species produced in all types of cells [44]. Evidence from Maziere's study suggested that OPN gene expression was augmented by oxidative stress as measured by intracellular levels of reactive oxygen species (ROS) and lipid peroxidation products in a dose- and time-dependent manner whatever the cell specificity [45]. Thus,

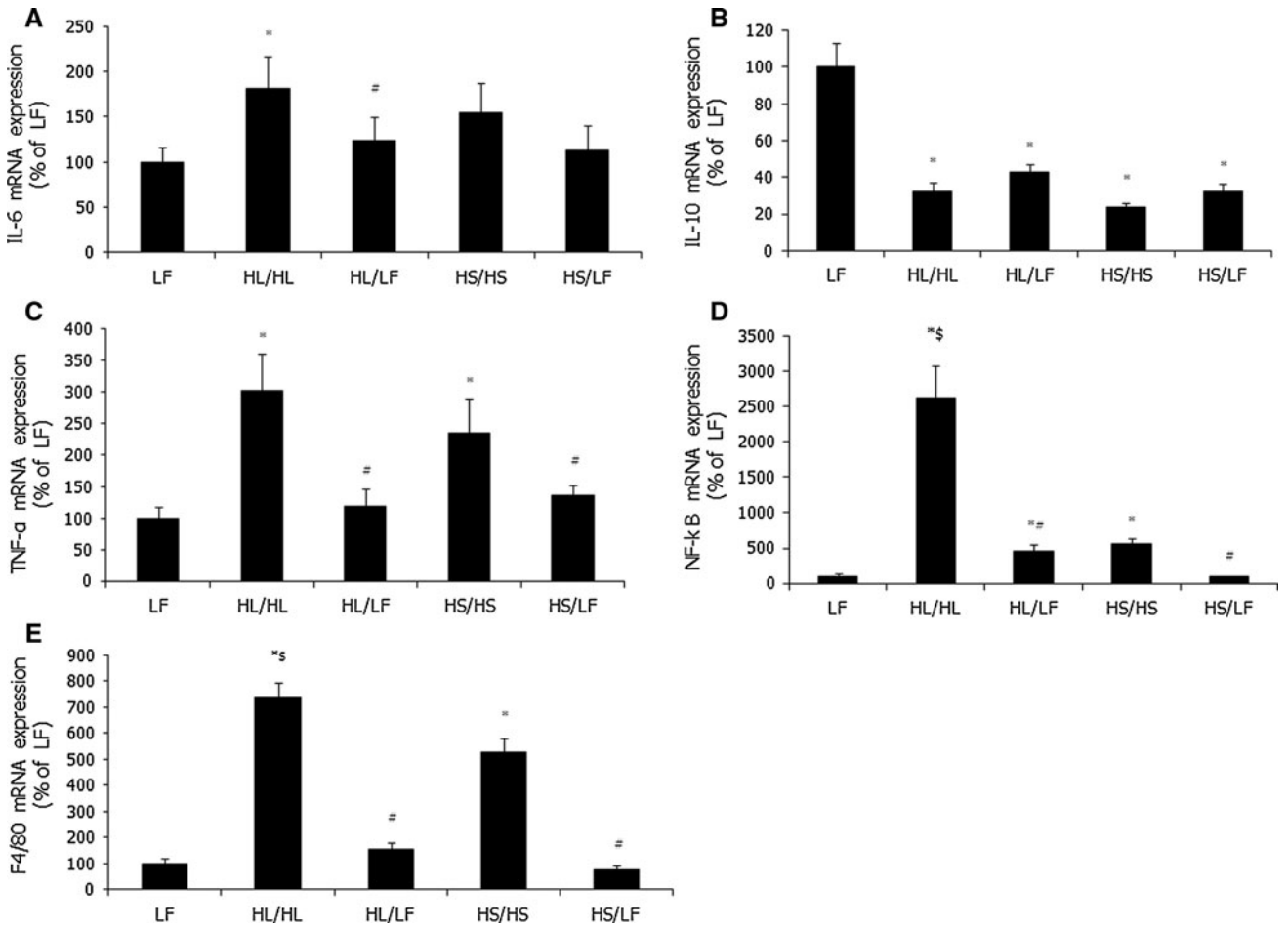

Fig. 3 Comparative effects of different diets on mRNA expressions of IL-6 (**a**), IL-10 (**b**), NF-κB (**c**) and F4/80 (**d**) in adipose tissue. Expression of mRNA of indicated genes relative to endogenous control of each sample was determined by quantitative real-time PCR. $n = 5$–6/group. Data (mean ± SEM) were given in percentage of the LF group. $^{*}P < 0.05$ versus LF group, $^{\#}P < 0.05$ versus corresponding group received respective continuous high-fat diet, $^{\$}P < 0.05$ versus corresponding group received HS

by decreasing endoplasmic reticulum stress state and oxidative stress level, PUFA may antagonize the upregulation of OPN expression by high-fat diet. Also, we could not exclude the contribution of bioactive compounds such as flavors and Vitamin E contained in soybean oil.

In summary, the enhanced OPN expression in high-fat diet-induced obese rats might be responsible for the activated adipose tissue macrophages, increased inflammatory genes expression and development of insulin resistance. Either replacing lard oil with soybean oil in high-fat diet or reducing dietary fat can attenuate the upregulation of OPN by high-fat diet. The molecular mechanisms underlying and specific component responsible for the differential effect of lard oil and soybean oil on OPN expression remain to be determined.

Acknowledgments This work was supported by National Natural Science Foundation of China (No. 30671765) and Scientific Research Foundation for Returned overseas Chinese Scholars, Ministry of Education of China (No. 20101561).

Conflict of interest The authors have declared no conflict of interest.

References

1. Dandona P, Aljada A, Bandyopadhyay A (2004) Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 25:4–7
2. Wellen KE, Hotamisligil GS (2003) Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 112:1785–1788
3. Shoelson SE, Herrero L, Naaz A (2007) Obesity, inflammation, and insulin resistance. Gastroenterology 132:2169–2180
4. Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, Giachelli CM (1998) NF-kappaB mediates alphavbeta3 integrin-induced endothelial cell survival. J Cell Biol 141:1083–1093
5. Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117:175–184
6. Bassaganya-Riera J, Misyak S, Guri AJ, Hontecillas R (2009) PPAR gamma is highly expressed in F4/80(hi) adipose tissue

macrophages and dampens adipose-tissue inflammation. Cell Immunol 258:138–146
7. Standal T, Borset M, Sundan A (2004) Role of osteopontin in adhesion, migration, cell survival and bone remodeling. Exp Oncol 26:179–184
8. Young MF, Kerr JM, Termine JD, Wewer UM, Wang MG, McBride OW, Fisher LW (1990) cDNA cloning, mRNA distribution and heterogeneity, chromosomal location, and RFLP analysis of human osteopontin (OPN). Genomics 7:491–502
9. Saitoh Y, Kuratsu J, Takeshima H, Yamamoto S, Ushio Y (1995) Expression of osteopontin in human glioma. Its correlation with the malignancy. Lab Invest 72:55–63
10. Weber GF, Cantor H (1996) The immunology of Eta-1/osteopontin. Cytokine Growth Factor Rev 7:241–248
11. Mazzali M, Kipari T, Ophascharoensuk V, Wesson JA, Johnson R, Hughes J (2002) Osteopontin—a molecule for all seasons. QJM 95:3–13
12. Xu G, Sun W, He D, Wang L, Zheng W, Nie H, Ni L, Zhang D, Li N, Zhang J (2005) Overexpression of osteopontin in rheumatoid synovial mononuclear cells is associated with joint inflammation, not with genetic polymorphism. J Rheumatol 32:410–416
13. Isoda K, Kamezawa Y, Ayaori M, Kusuhara M, Tada N, Ohsuzu F (2003) Osteopontin transgenic mice fed a high-cholesterol diet develop early fatty-streak lesions. Circulation 107:679–681
14. Ramaiah SK, Rittling S (2008) Pathophysiological role of osteopontin in hepatic inflammation, toxicity, and cancer. Toxicol Sci 103:4–13
15. Kiefer FW, Zeyda M, Todoric J, Huber J, Geyeregger R, Weichhart T, Aszmann O, Ludvik B, Silberhumer GR, Prager G, Stulnig TM (2008) Osteopontin expression in human and murine obesity: extensive local up-regulation in adipose tissue but minimal systemic alterations. Endocrinology 149:1350–1357
16. Gomez-Ambrosi J, Catalan V, Ramirez B, Rodriguez A, Colina I, Silva C, Rotellar F, Mugueta C, Gil MJ, Cienfuegos JA, Salvador J, Fruhbeck G (2007) Plasma osteopontin levels and expression in adipose tissue are increased in obesity. J Clin Endocrinol Metab 92:3719–3727
17. Chapman J, Miles PD, Ofrecio JM, Neels JG, Yu JG, Resnik JL, Wilkes J, Talukdar S, Thapar D, Johnson K, Sears DD (2010) Osteopontin is required for the early onset of high fat diet-induced insulin resistance in mice. PLoS One 5:e13959
18. Nomiyama T, Perez-Tilve D, Ogawa D, Gizard F, Zhao Y, Heywood EB, Jones KL, Kawamori R, Cassis LA, Tschop MH, Bruemmer D (2007) Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. J Clin Invest 117:2877–2888
19. Kiefer FW, Zeyda M, Gollinger K, Pfau B, Neuhofer A, Weichhart T, Saemann MD, Geyeregger R, Schlederer M, Kenner L, Stulnig TM (2010) Neutralization of osteopontin inhibits obesity-induced inflammation and insulin resistance. Diabetes 59:935–946
20. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ (2000) Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 85:2402–2410
21. Mather K (2009) Surrogate measures of insulin resistance: of rats, mice, and men. Am J Physiol Endocrinol Metab 296:E398–E399
22. Li S, Zhang HY, Hu CC, Lawrence F, Gallagher KE, Surapaneni A, Estrem ST, Calley JN, Varga G, Dow ER, Chen Y (2008) Assessment of diet-induced obese rats as an obesity model by comparative functional genomics. Obesity (Silver Spring) 16:811–818
23. Tschop M, Heiman ML (2001) Rodent obesity models: an overview. Exp Clin Endocrinol Diabetes 109:307–319
24. Van Heek M, Compton DS, France CF, Tedesco RP, Fawzi AB, Graziano MP, Sybertz EJ, Strader CD, Davis HR Jr (1997) Diet-induced obese mice develop peripheral, but not central, resistance to leptin. J Clin Invest 99:385–390
25. Buettner R, Scholmerich J, Bollheimer LC (2007) High-fat diets: modeling the metabolic disorders of human obesity in rodents. Obesity (Silver Spring) 15:798–808
26. Levin BE, Dunn-Meynell AA, Balkan B, Keesey RE (1997) Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. Am J Physiol 273:R725–R730
27. Paulino G, Barbier de la Serre C, Knotts TA, Oort PJ, Newman JW, Adams SH, Raybould HE (2009) Increased expression of receptors for orexigenic factors in nodose ganglion of diet-induced obese rats. Am J Physiol Endocrinol Metab 296:E898–E903
28. Wang C, Yang N, Wu S, Liu L, Sun X, Nie S (2007) Difference of NPY and its receptor gene expressions between obesity and obesity-resistant rats in response to high-fat diet. Horm Metab Res 39:262–267
29. Ruzickova J, Rossmeisl M, Prazak T, Flachs P, Sponarova J, Veck M, Tvrzicka E, Bryhn M, Kopecky J (2004) Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue. Lipids 39:1177–1185
30. Belzung F, Raclot T, Groscolas R (1993) Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. Am J Physiol 264:R1111–R1118
31. Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Scholmerich J, Bollheimer LC (2006) Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. J Mol Endocrinol 36:485–501
32. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112:1821–1830
33. Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444:860–867
34. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808
35. Fain JN (2006) Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm 74:443–477
36. Bertola A, Deveaux V, Bonnafous S, Rousseau D, Anty R, Wakkach A, Dahman M, Tordjman J, Clement K, McQuaid SE, Frayn KN, Huet PM, Gugenheim J, Lotersztajn S, Le Marchand-Brustel Y, Tran A, Gual P (2009) Elevated expression of osteopontin may be related to adipose tissue macrophage accumulation and liver steatosis in morbid obesity. Diabetes 58:125–133
37. Weber GF, Zawaideh S, Hikita S, Kumar VA, Cantor H, Ashkar S (2002) Phosphorylation-dependent interaction of osteopontin with its receptors regulates macrophage migration and activation. J Leukoc Biol 72:752–761
38. Zeyda M, Gollinger K, Todoric J, Kiefer FW, Keck M, Aszmann O, Prager G, Zlabinger GJ, Petzelbauer P, Stulnig TM (2011) Osteopontin is an activator of human adipose tissue macrophages and directly affects adipocyte function. Endocrinology 152:2219–2227
39. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red Eagle A, Vats D, Brombacher F, Ferrante AW, Chawla A (2007) Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature 447:1116–1120
40. Stienstra R, Duval C, Keshtkar S, van der Laak J, Kersten S, Muller M (2008) Peroxisome proliferator-activated receptor gamma activation promotes infiltration of alternatively activated macrophages into adipose tissue. J Biol Chem 283:22620–22627

41. Flachs P, Horakova O, Brauner P, Rossmeisl M, Pecina P, Franssen-van Hal N, Ruzickova J, Sponarova J, Drahota Z, Vlcek C, Keijer J, Houstek J, Kopecky J (2005) Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. Diabetologia 48:2365–2375
42. Arai T, Kim HJ, Chiba H, Matsumoto A (2009) Anti-obesity effect of fish oil and fish oil-fenofibrate combination in female KK mice. J Atheroscler Thromb 16:674–683
43. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306:457–461
44. Cullinan SB, Diehl JA (2006) Coordination of ER and oxidative stress signaling: the PERK/Nrf2 signaling pathway. Int J Biochem Cell B 38:317–332
45. Maziere C, Gomila C, Maziere JC (2010) Oxidized low-density lipoprotein increases osteopontin expression by generation of oxidative stress. Free Radical Bio Med 48:1382–1387