

Effects of dietary Chinese cured meat on lipid metabolism in rats

Mingmin Xiong^a, Yumei Zhang^b, Xianbiao Li^b, Changwei Ma^{a,*}

^a College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, 100083, PR China

^b Department of Nutrition and Food Hygiene, Public Health School, Peking University, Beijing 100083, PR China

Received 10 January 2007; received in revised form 10 May 2007; accepted 12 July 2007

Abstract

The effects of different fats on animal lipid metabolism were investigated in order to clarify the safety of cured meat. The physical effect of nitrite in the cured meat was also explored due to a increased concern of this compound as a food additive. Body weight, food intake, organ/body weight ratio and plasma lipid profiles were analyzed. Rats fed with cured meat fat did not show any differences in body weight and food intake, compared with the control, and no significant difference was observed among groups. However, lard-fed rats showed marked morphological alternations in aortic intima and liver tissue. The cured meat group did not show apparent morphological changes on aorta intima compared to the control. Rats that received lard diet had significantly elevated TC (2.27 ± 0.46 mmol/ml), higher LDL-C (0.78 ± 0.31 mmol/ml) but lower HDL-C (1.55 ± 0.14 mmol/ml). Lard-fed rats had elevated oxidized LDL in serum and MDA level in blood and liver. However, diets containing fresh fat (with/without added nitrite) or cured meat fat failed to show these detrimental effects.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Chinese cured meat; Organ/body weight ratio; Plasma lipid profile; Lipid oxidation

1. Introduction

Hypercholesterolemia is a major causative factor in atherogenesis (Steinberg, 2002, 2005). In humans, intake of excessive energy from an energy-condensed diet with high percentage of fat is associated with increased incidence of cardiovascular disease, diabetes and certain cancers, and it is also a major cause of disability and death in industrialized countries (Willett, 2000). Epidemiological evidence and cohort studies have suggested a positive correlation between fat intake and incidences of breast, colon and prostate cancer (Armstrong & Doll, 1975; Fleshner, Bagnell, Klotz, & Venkateswaran, 2004; Oba et al., 2006; Sasaki, Horacsek, & Kesteloot, 1993). Weight gain, induced by high-fat feeding, was involved in increased liver oxidative stress (Milagro, Campion, & Martinez, 2006). In addition, long-term high fat diet also triggered chronic pancreatic

injuries (Yan, Li, Meng, Ren, & Kou, 2006). Animal studies have suggested that consumption of a high fat diet increased adiposity and shortened mammary tumor latency (Cleary, Grande, & Maihle, 2004), and specifically, a maternal diet high in fat during the fetal period of pregnancy was sufficient to increase reproductive system tumors and metastases in female offspring (Walker & Kurth, 1995).

Chinese cured meat is one of the traditional cured products, and the food has been popular for centuries due to its special colour and taste, resulting from its unique processing. The cured meat is made from fresh pork, cured with salt, crushed aniseed, dark soy sauce and then air-dried or baked. Sodium nitrite is most commonly used for curing, which has raised concern about its consumption, due to incidence of various cancers in the past two decades (Fraser, Chilvers, Beral, & Hill, 1980). Epidemiological evidence shows some correlation between consumption of cured meats during pregnancy and the subsequent risk of brain tumors, as well as other cancers, in the offspring (Klurfeld, 2001; Pogoda & Preston-Martin, 2001).

* Corresponding author. Fax: +86 10 62737643.

E-mail address: chwma@cau.edu.cn (C. Ma).

However, earlier reports indicate that male mice orally administered with nitrite showed neither significant changes in body weight nor any other physical functions at a dose of 110 mg/kg or less (Kinoshita & Kakihira, 1982). In recent years, new debate has arisen on whether nitrite is detrimental or beneficial to human health. McKnight, Duncan, Leifert, and Golden (1999) and Archer (2002) observed that nitrite in diet could act as an effective host defence agent against gastrointestinal pathogens and a modulator for platelet activity.

Many previous reports have been focussed on characteristics of cured meat and the development of curing technology, but little on the physiological effects of cured meat on rat *in vivo*. Therefore, our objectives were to determine the effects of cured meat fat on rats: the effect of fat from cured meat vs. fresh meat on animal lipid metabolism and the physical effect of nitrite *per se* vs. nitrite in the cured meat on the rats.

2. Materials and methods

2.1. Preparation of fresh fat, lard and Chinese cured meat fat

The lard was rendered from fresh fat. The fresh fat tissue, from under the skin, was passed through a meat grinder and heated in a large, shallow pot. Liquid lard was continually squeezed from the raw fat and collected to be used for the preparation of rat diets.

Fresh pork samples were purchased from local market. The meat was rinsed and sliced into bony ribs with a thickness of 4–5 cm and each piece weighed about 1 kg. Dried cassia bark, aniseed and pepper were mixed and crushed. The meat slices were subjected to curing by soaking in the pickle solution with 7 kg salt, 0.2 kg nitrite and 0.4 kg crushed spices, for 15–18 h. Then the pork slices were taken out, rinsed to remove excessive salt and hung up to dry. The dried slices were hung in a smoking house initiated by 8–9 kg charcoal and 12 kg wood chips for 28 h and air-dried for 3–4 months. The cured meat fat for experiment was excised from the mature cured meat and minced for the preparation of rat diets.

2.2. Physical properties of the Chinese cured meat fat

Melting point of the cured meat fat was measured on an X-5 micro-melting point detector (Shanghai Guangying Instrument Factory, Shanghai, China); 0.05 mg of cured meat fat and fresh fat were frozen at -20°C , minced and their melting points measured using the detector. The melting points were observed by gradually increasing the temperature from 35°C to 50°C for the fresh fat and from 36°C to 51°C for the Chinese cured meat fat.

2.3. Animal diets and sample collection

Twelve-week old male Sprague–Dawley rats were purchased from the Animal Experiment Center, Medical Col-

lege of Beijing University and housed individually in screen bottomed stainless steel cages in a room with controlled temperature ($23 \pm 2^{\circ}\text{C}$), light (12:12 h light \pm dark cycle) and humidity (60%). All were fed a basal diet of AIN-93G, as described by Reeves (1997), containing (percent by weight) cornstarch 39.75, casein 20.00, sucrose 10.00, cellulose fibre 5.0, corn oil 7.0, AIN-93G-MX mineral mixture 3.5, AIN-93G-MX vitamin mixture 1.0, L-cystine 0.3, choline bitartrate 0.25. The rats were then randomly assigned to eight groups with eight rats each. Each group was fed with the following diet: Group I, basal diet (AIN-93G); Group II, AIN-93G + 1% cholesterol + 0.3% bile salt (pig) + 10% lard; group III, AIN-93G + 5% fresh fat; group IV, AIN-93G + 10% fresh fat; group V, AIN-93G + 10% fresh fat + 0.3% nitrite; group VI, AIN-93G + 5% cured meat fat; group VII, AIN-93G + 10% cured meat fat; group VIII, AIN-93G + 20% cured meat fat. Food intake and body weight were monitored daily during the study. Rat tails were taken every 4 weeks to measure plasma lipid. After 8 weeks, all rats were killed by cervical dislocation. During necropsy, heart, liver, spleen, kidneys, testicle, peri-testis brown fat tissues, abdominal aorta and 10 cm of superior segment of jejunal were excised, weighed and quickly frozen in liquid nitrogen. All samples were kept at -80°C for further assays.

2.4. Histopathological analysis

Liver and aorta tissues were cut into sections of 1 mm, fixed in formalin and embedded in paraffin blocks (glass slide was cleaned with 95% ethanol, treated with APES solution and air-dried) using microtome and applied to slides. Hematoxylin and eosin (HE) staining was performed according to the standard procedure.

2.5. Plasma lipid analysis

Blood samples were collected from the rat tail vein into chilled paraoxon-coated capillary tubes on weeks 2, 4, 6 and 8, as described previously (Zambon, Hashimoto, & Brunzell, 1993). Plasma lipid profiles in terms of total cholesterol, LDL-cholesterol, HDL-cholesterol and TG, were assessed by enzymatic colorimetric assay, using commercially available reagent kits (Benado et al., 2004; Qader, Salehi, Hakanson, Lundquist, & Ekelund, 2005). LDL-cholesterol values were obtained through Friedewald formula (Johnson, McNutt, MacMahon, & Robson, 1997).

Oxidized LDL was measured by determining the level of baseline diene conjugation in lipids extracted from LDL (Vasankari et al., 2001). In brief, serum LDL was isolated by precipitation with buffered heparin. Lipids were extracted from LDL samples by chloroform–methanol, dried under nitrogen, then re-dissolved in cyclohexane and analyzed spectrophotometrically at 234 nm.

The concentration of lipid oxidation products-malonaldehyde (MDA) in the plasma and liver was determined, based on the measurement described in the literature

(Moore & Roberts, 1998). The results were expressed in nmols/ml of plasma. Each experiment was performed in triplicate.

2.6. Statistical analysis

The data were expressed as means \pm SD. Statistical calculations were performed using a statistical software package (SPSS, version 13.0; SPSS Inc; Chicago, IL). Statistically significant differences were analyzed by parametric or non-parametric tests when required. Analysis of variance with the Bonferroni/Dunn multicomparison method as a *post hoc* test was used for intragroup comparison (i.e., Tukey test). A *p* value of <0.05 indicated significance.

3. Results

3.1. The effect of various fats on body weight and food intake

The growth patterns of rats are shown in Table 1. During the feeding period, the weight gains of all groups were nearly identical and no significant differences were found among groups. The body weights increased stably with time. However, from the 4th week, the growth speed was

reduced. Food intakes of all groups are shown in Table 2. Marked difference in food intake was observed in the first 4 weeks between the basal diet group and fresh fat or cured meat fat group. However, it was not possible to observe this after 4 weeks. Combining the body weight with food intake, it was possible to see that diets of fresh fat and cured meat fat at different dose did not have any effect on body weights compared to the basal diet.

3.2. The effect of various fat on ratios of organ/body weight

As shown in Table 3, except for the ratio of heart/body, testicle/body and kidney/body, ratios of liver/body weight and spleen/body weight in groups administered with 10% lard were found to be significantly different from the other groups. At the same fat dose, lard resulted in pronounced elevation in ratios of liver/body and spleen/body. Compared to control, administration of fresh fat and cured meat fat did not result in any alternations of organs in SD rats.

3.3. Morphological studies on aorta and liver

The surface structures of aortic intima derived from rats fed with basal diet are illustrated in Fig. 1a. It can be seen

Table 1
Influence of fresh fat and cured meat fat on body weight of male SD rat following 4-weeks feeding

Groups	Body weight (g)				
	0 week	2 weeks	4 weeks	6 weeks	8 weeks
Group I (Control, AIN-93G)	259.25 \pm 3.69*	374.75 \pm 19.18	493.8 \pm 42.04	517.25 \pm 49.18	535.50 \pm 56.48
Group II (AIN-93G + 1% cholesterol + 0.3% bile salt (pig) + 10% lard fat)	260.25 \pm 5.59	378.50 \pm 37.32	490.88 \pm 69.89	514.06 \pm 72.84	526.88 \pm 73.41
Group III (AIN-93G + 5% fresh fat tissue)	262.00 \pm 5.95	378.00 \pm 26.73	489.38 \pm 40.63	507.13 \pm 49.00	522.75 \pm 50.85
Group IV (AIN-93G + 10% fresh fat tissue)	260.63 \pm 5.85	383.38 \pm 9.05	504.63 \pm 36.90	527.19 \pm 36.59	547.38 \pm 40.58
Group V (AIN-93G + 10% fresh fat tissue + 0.3% nitrite)	257.88 \pm 3.79	376.88 \pm 22.19	484.25 \pm 56.69	506.63 \pm 63.38	520.25 \pm 67.33
Group VI (AIN-93G + 5% cured meat fat)	261.75 \pm 6.67	375.13 \pm 25.51	463.88 \pm 30.38	495.13 \pm 62.15	511.00 \pm 67.77
Group VII (AIN-93G + 10% cured meat fat)	259.25 \pm 5.34	374.13 \pm 23.27	486.38 \pm 40.21	502.13 \pm 36.14	519.75 \pm 40.09
Group VIII (AIN-93G + 20% cured meat fat)	256.38 \pm 3.89	380.25 \pm 14.80	489.13 \pm 26.32	510.44 \pm 33.93	529.75 \pm 37.98

* Each value represents the mean \pm SD of eight mice/group. Within the same column, means followed by different letters are significantly different at $p < 0.05$.

Table 2
Influence of fresh fat and cured meat fat on body weight of male SD rat following 8-weeks feeding

Groups	Daily food intake (g)			
	2 weeks	4 weeks	6 weeks	8 weeks
Group I (Control, AIN-93G)	33.69 \pm 2.44*	19.75 \pm 2.68	23.52 \pm 2.16	21.98 \pm 2.11
Group II (AIN-93G + 1% cholesterol + 0.3% bile salt (pig) + 10% lard fat)	28.94 \pm 2.78	20.93 \pm 2.70	24.17 \pm 2.19	23.00 \pm 2.37
Group III (AIN-93G + 5% fresh fat tissue)	32.47 \pm 5.01	19.08 \pm 1.71	23.50 \pm 2.44	22.56 \pm 3.18
Group IV (AIN-93G + 10% fresh fat tissue)	31.63 \pm 2.66	23.55 \pm 1.67b	24.13 \pm 2.27	25.19 \pm 1.95
Group V (AIN-93G + 10% fresh fat tissue + 0.3% nitrite)	34.88 \pm 5.12	23.60 \pm 2.27b	25.00 \pm 1.58	23.08 \pm 3.07
Group VI (AIN-93G + 5% cured meat fat)	29.41 \pm 3.17a	17.60 \pm 3.01a	23.23 \pm 2.76	21.81 \pm 3.76
Group VII (AIN-93G + 10% cured meat fat)	29.66 \pm 3.24	16.83 \pm 1.52a	23.29 \pm 1.70	22.00 \pm 2.52
Group VIII (AIN-93G + 20% cured meat fat)	28.50 \pm 4.39b	21.95 \pm 2.73	23.13 \pm 2.33	23.25 \pm 2.19

* Each value represents the mean \pm SD of eight mice/group. Within the same column, means followed by different letters are significantly different at $p < 0.05$.

Table 3
Influence of dietary fresh fat tissue and cured meat fat on organ/body weight in SD rat

Groups	Organ/body weight (g/kg)				
	Heart/weight	Liver/weight	Spleen/weight	Kidney/weight	Testicle/weight
Group I (Control)	3.1 ± 0.3*	26.4 ± 3.6a	1.5 ± 0.2a	9.7 ± 0.1	6.4 ± 0.6
Group II (AIN-93G + 1% cholesterol + 0.3% bile salt (pig) + 10% lard fat)	3.2 ± 0.3	44.1 ± 4.7b	2.1 ± 0.8b	6.5 ± 0.4	6.2 ± 0.9
Group III (AIN-93G + 5% fresh fat)	3.5 ± 0.04	25.9 ± 2.6a	1.4 ± 0.2a	7.1 ± 0.5	7.1 ± 0.8
Group IV (AIN-93G + 10% fresh fat)	3.2 ± 0.05	25.2 ± 2.2a	1.5 ± 0.2a	6.3 ± 0.6	5.9 ± 1.0
Group V (AIN-93G + 10% fresh fat + 0.3% nitrite)	3.5 ± 0.04	24.8 ± 4.8a	1.5 ± 0.3a	7.3 ± 0.4	6.8 ± 0.8
Group VI (AIN-93G + 5% cured meat)	3.1 ± 0.03	25.4 ± 1.8a	1.4 ± 0.2a	6.9 ± 0.4	7.2 ± 0.9
Group VII (AIN-93G + 10% cured meat)	3.4 ± 0.04	26.3 ± 2.0a	1.5 ± 0.2a	6.8 ± 0.8	6.3 ± 0.4
Group VIII (AIN-93G + 20% cured meat)	3.1 ± 0.05	26.2 ± 2.8a	1.5 ± 0.3a	6.2 ± 0.7 ^b	6.4 ± 0.7

* Each value represents the mean ± SD of eight mice/group. Within the same column, means followed by different letters are significantly different at $p < 0.05$.

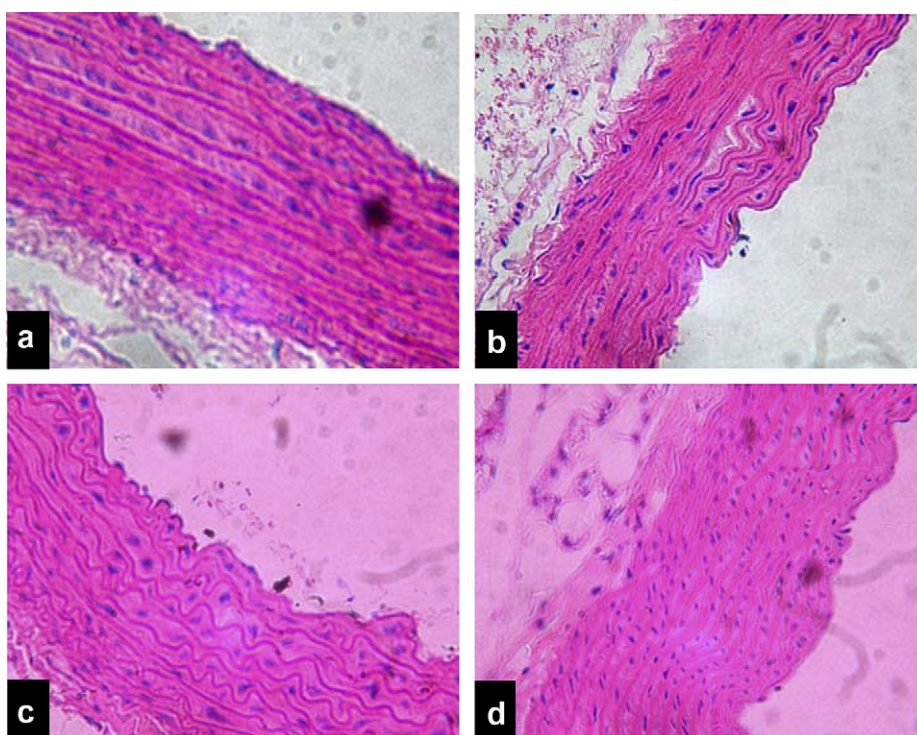


Fig. 1. Effect of cured meat fat and fresh fat administration on endothelium derived from rat aortas. (a) Control; (b) lard fat; (c) fresh fat(10%); and (d) cured meat fat (10%).

that the endothelial cells are arranged intensively without abnormal proliferation. In SD rats administered with pork lard, however, the aortic intima were found to be incrassate and endothelia injury is clearly seen in Fig. 1b. The transformation of foam cells is visible and cholesterol was deposited in the inner wall of the aorta. The inner aortic wall was also observed to be uneven and there were fibrosis and atherosclerotic plaques. Lipid infiltration was present in all layers of aortic wall. Compared to the control group, tissues from rats fed with fresh fat were slightly thinner (Fig. 1c). Rats fed with cured meat at 10% fat did not demonstrate virtual morphological alterations on aortic intima. Similar to the control, the main inner part of the aortic walls was smooth (Fig. 1d).

The effect of high fat on the morphology of hepatocytes is shown in Fig. 2. Hepatocytes derived from the control group were observed to have complete and distinct nucleus (Fig. 2a). In rats fed with 10% lard, the morphological alternations of hepatocytes were quite obvious. Microscopic observations indicated that hepatic cells were fat-storing and much incomplete in shape, with their nucleus roughly crushed to the edge. In addition, a large proportion of hepatic cells were present in white. Surprisingly, only a slight fat-storing was found in the hepatocytes of the cured meat fed rats and the hepatocytes still had an integrated appearance (Fig. 2d). Hepatic cells of rats fed with fresh fat showed fewer morphological alternations than did those fed with lard,

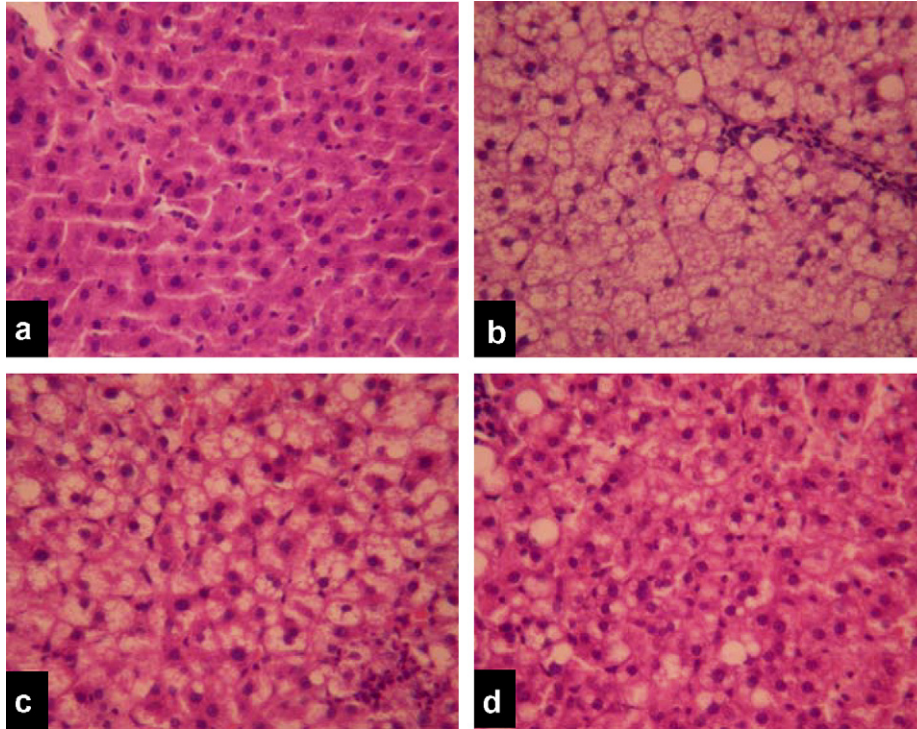


Fig. 2. Effect of cured meat fat and fresh fat administration on rat liver. (a) Control; (b) lard fat; (c) fresh fat(10%); and (d) cured meat fat (10%).

showing slightly crushed nucleus and fat-deposit (Fig. 2c).

3.4. Effect of different diet on plasma lipid profiles

The plasma levels of triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C), determined on the 4th week and 8th week in all groups, are presented in Fig. 3. Plasma triglyceride levels decreased in all groups, and the most significant reduction on the plasma triglyceride level was observed in the lard-fed group. Administration of lard and fresh fat plus nitrite markedly lowered serum TG. No significant difference of TG was observed between fresh fat and cured meat fat. Cured meat fat feeding resulted in a reduction of serum total cholesterol (TC) while lard fat or fresh fat did not. No significant difference was found among groups except that rats fed with 10% lard had the highest LDL-C of 0.78 ± 0.31 mM in blood, which was significantly different from the other groups ($p < 0.05$). Compared to the control, all high-fat diets except for 10% fresh fat led to a drop in serum HDL-C.

LDL-C in rats showed a similar pattern after feeding for 8 weeks. The highest level of LDL-C was observed in the group fed with lard fat, while the levels of LDL-C in groups fed with either fresh fat or Chinese cured meat fat diet, at various dosages, were similar to the control. Com-

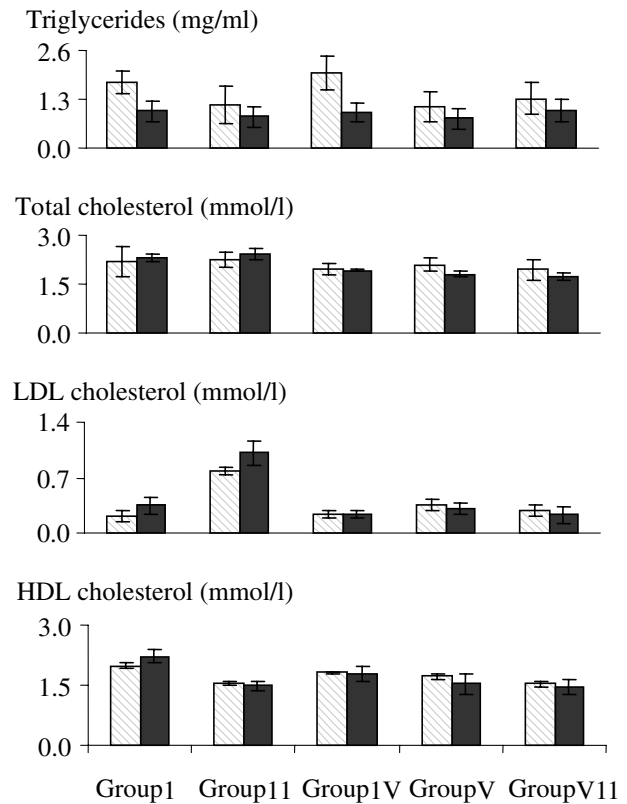


Fig. 3. Plasma lipid profile of rats that received various diets with fat contents at 10%. ▨ 4 weeks; ■ 8 weeks.

Table 4
Effects of dietary fresh fat tissue and cured meat fat on the level of serum Ox-LDL and MDA in serum and liver

Group	Serum Ox-LDL ($\mu\text{g}/\text{dl}$)	Serum MDA (nmol/ml)	Liver MDA (nmol/ml)
Group I (Control)	20.3 \pm 3.74ac	4.27 \pm 1.92a	5.13 \pm 0.71a
Group II (AIN-93G + 1% cholesterol + 0.3% bile salt (pig) + 10% lard fat)	40.5 \pm 4.23b	7.67 \pm 1.65b	8.56 \pm 1.69b
Group III (AIN-93G + 5% fresh fat)	24.5 \pm 4.13a	5.32 \pm 0.61a	5.963 \pm 1.23a
Group IV (AIN-93G + 10% fresh fat)	27.1 \pm 4.38a	5.51 \pm 1.07a	6.05 \pm 0.34a
Group V (AIN-93G + 10% fresh fat + 0.3% nitrite)	23.4 \pm 2.65a	5.24 \pm 0.88a	5.83 \pm 0.35a
Group VI (AIN-93G + 5% cured meat)	24.5 \pm 3.33a	5.45 \pm 1.16a	5.39 \pm 0.63a
Group VII (AIN-93G + 10% cured meat)	31.0 \pm 3.61a	5.89 \pm 1.71ab	6.21 \pm 0.88ab
Group VIII (AIN-93G + 20% cured meat)	31.7 \pm 4.89abc	5.90 \pm 1.24ab	6.83 \pm 1.83ab

* Each value represents the mean \pm SD of eight mice/group. Within the same column, means followed by different letters are significantly different at $p < 0.05$.

pared to the plasma lipid level at the 4th week, TG in all groups at the 8th week was lowered while other lipids remained more or less unchanged ($p > 0.05$) (Fig. 3).

3.5. The level of serum oxidation

LDL is important to maintain the structural integrity of cells; when oxidized it can trigger inflammation, which in turn can lead to coronary artery disease. As shown in Table 4, oxidized-LDL value in the lard-fed group was significantly higher than in groups fed with fresh fat or cured meat fat ($p < 0.05$). In both cases, 4 weeks and 8 weeks, oxidized LDL was significantly related to total plasma LDL-C ($\lambda = 0.70$ and $\lambda = 0.695$, $p < 0.05$) while HDL-C showed negative relation to HDL cholesterol ($\lambda = -0.638$ and $\lambda = -0.650$, $p < 0.05$).

The levels of MDA in serum and liver are important indicators of the oxidation of lipid. MDA levels in blood and liver are shown in Table 4. Rats given the diet of lard had significantly higher MDA values in serum and liver than did those given other treatments ($p < 0.05$). The fresh fat or cured meat fat did not affect serum or hepatic MDA levels.

4. Discussion

Sodium nitrite is commonly used for curing due to its inhibitory role against spores of *Clostridium botulinum* and other microorganisms, as well as against oxidation, thus maintaining an attractive appearance and fresh flavour (Shahidi, Synowiecki, & Sen, 1992). Much concern has been raised about the safety of cured meat due to its containing nitrite as a preservative. Meanwhile, the growing consumption of meat with high-fat content has been reported to be associated with heart disease, strokes and cancers (Giovannucci et al., 1994; Kolonel, 2001). Thus, it is necessary to check the physiological effect of cured meat diet on rats and its consumption safety as well. Lard, fresh fat and cured meat fat, at varying doses, were applied to see whether cured meat specifically altered plasma lipid profile in rats. A combined diet, with nitrite added to fresh fat, was also adopted to explore whether there was difference between real cured meat and the mimicked one.

Results indicated that feeding rats with forms of fresh fat, lard and cured meat at doses of 5%, 10% and 20% fat showed no significant differences in rat body weight or food intake. However, as shown in Table 3, the masses of liver and spleen were much higher in the lard-fed group than in the other groups. The ratio of organ to body weight in lard-fed rats was significantly higher ($p < 0.05$) than those of the control, fresh fat fed, and cured meat fat fed rats. These data suggested that a cured meat diet at the dose of 5%, 10% and 20% did not cause any alterations of body weight and food intake or organ/body weight ratios. In particular, we found that, even in the presence of 0.3% nitrite, rats fed with fresh fat also showed no difference from the control.

Triglyceride (TG) levels (and its subclasses) are independent predictors of hypertriglyceridemia (Hokanson & Austin, 1996; Miller, Seidler, Moalemi, & Pearson, 1998). In this study, we did not observe the difference of TG level caused by various forms of diets. However, the total cholesterol level in the rats fed with cured meat fat was significantly different from those in the control or those fed with lard or fresh fat. Compelling evidence, from meta-analysis of a number of clinical studies on a large aggregate of patients, has established an increased level of triglycerides as an independent risk factor for cardiovascular disease (Johnson et al., 1997) and high cholesterol levels are now associated with heart disease as well (Stein, Weinstein, Stein, & Steinberg, 1976). However, in our study, cured meat, even at high dose of 20%, was able to lower the serum TC and had a protective effect on artery. High LDL-C is also a risk factor for coronary heart disease while high HDL-C is helpful in transporting excess cholesterol to the liver for excretion in the bile (Ansell, Watson, Fogelman, Navab, & Fonarow, 2005; Bruce, Chouinard, & Tall, 1998; Phillips et al., 1998). So it is not surprising to find that lard diet is able to significantly increase serum LDL-C while LDL-C value in the cured meat fed group is not significantly different from the control and the group fed with fresh fat. However, both lard and cured meat fat diet could reduce the HDL-C level, which was detrimental for cardiovascular health (Li et al., 2004). This result was further confirmed by morphological studies on cardiovascular tissues and liver.

Oxidatively modified low density lipoprotein (LDL) activates endothelial cells, leading to an alteration of the functional and structural integrity of the endothelial barrier (Berliner et al., 1995; Ross, 1993; Steinberg, 1997). In lard-fed rats, the intima of aorta displayed significant morphological alterations. Further analysis on serum oxidized LDL-C revealed that this morphological alteration of aortic intima was closely correlated with elevated Ox-LDL in lard-fed group. Cured meat-fat-fed rats, at doses of 5%, 10% and 20%, showed no difference from the control or fresh fat fed groups. Elevated MDA levels in blood and liver were also closely correlated with serum Ox-LDL, which indicated that serious lipid oxidation in endothelial cells could lead to the formation of fibrosis and atherosclerotic plaques on the aortic wall. We also found that, among these groups, HDL-C values were negatively correlated with Ox-LDL. These results were also consistent with studies that HDL-C was able to inhibit the formation of oxidized LDL (Navab, Hama, Anantharamaiah et al., 2000; Navab, Hama, Cooke et al., 2000). In our study, rats that received cured meat diet did not experience any negative effect on the rat cardiovascular systems. Cured meat, with addition of nitrite during curing exerted the same effect on rat lipid metabolism as did fresh fat plus nitrite. Our research suggests that rising concerns about consumption of cured meats may not be justified, but an extensive investigation is required before a final conclusion can be drawn.

Acknowledgements

This work was supported by the international cooperation fund entitled “AMP-Activated Protein Kinase in Muscle Growth and Meat Quality” from the National Natural Science Foundation of China (NSFC-30540420523) and by the fund of National Key Technologies R&D Program of the Ministry of Science and Technology of China under 2006BAD05A03.

References

- Ansell, B. J., Watson, K. E., Fogelman, A. M., Navab, M., & Fonarow, G. C. (2005). High-density lipoprotein function recent advances. *Journal of the American College of Cardiology*, 46(10), 1792–1798.
- Archer, D. L. (2002). Evidence that ingested nitrate and nitrite are beneficial to health. *Journal of Food Protection*, 65(5), 872–875.
- Armstrong, B., & Doll, R. (1975). Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *International Journal of Cancer*, 15(4), 617–631.
- Benado, M., Alcantara, C., de la Rosa, R., Ambrose, M., Mosier, K., & Kern, M. (2004). Effects of various levels of dietary fructose on blood lipids of rats. *Nutrition Research*, 24(7), 565–571.
- Berliner, J. A., Navab, M., Fogelman, A. M., Frank, J. S., Demer, L. L., Edwards, P. A., et al. (1995). *Atherosclerosis: Basic mechanisms. Oxidation, inflammation, and genetics*. *Circulation*, 91(9), 2488–2496.
- Bruce, C., Chouinard, R. A., Jr., & Tall, A. R. (1998). Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. *Annual Review of Nutrition*, 18, 297–330.
- Cleary, M. P., Grande, J. P., & Maihle, N. J. (2004). Effect of high fat diet on body weight and mammary tumor latency in MMTV-TGF- α mice. *International Journal of Obesity and Related Metabolism Disorder*, 28(8), 956–962.
- Fleshner, N., Bagnell, P. S., Klotz, L., & Venkateswaran, V. (2004). Dietary fat and prostate cancer. *Journal of Urology*, 171(2 Pt 2), S19–S24.
- Fraser, P., Chilvers, C., Beral, V., & Hill, M. J. (1980). Nitrate and human cancer: a review of the evidence. *International Journal of Epidemiology*, 9(1), 3–11.
- Giovannucci, E., Rimm, E. B., Stampfer, M. J., Colditz, G. A., Ascherio, A., & Willett, W. C. (1994). Intake of fat, meat, and fiber in relation to risk of colon cancer in men. *Cancer Research*, 54(9), 2390–2397.
- Hokanson, J. E., & Austin, M. A. (1996). Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: A meta-analysis of population-based prospective studies. *Journal of Cardiovascular Risk*, 3(2), 213–219.
- Johnson, R., McNutt, P., MacMahon, S., & Robson, R. (1997). Use of the Friedewald formula to estimate LDL-cholesterol in patients with chronic renal failure on dialysis. *Clinical Chemistry*, 43(11), 2183–2184.
- Kinoshita, S., & Kakihira, H. (1982). The influence of sodium nitrite upon the physical functions of mice. *Sangyo Igaku*, 24(5), 471–474.
- Klurfeld, D. M. (2001). Maternal cured meat consumption during pregnancy and risk of paediatric brain tumour in offspring: potentially harmful levels of intake. *Public Health Nutrition*, 4(6), 1303–1305.
- Kolonel, L. N. (2001). Fat, meat, and prostate cancer. *Epidemiologic Reviews*, 23(1), 72–81.
- Li, J. Z., Chen, M. L., Wang, S., Dong, J., Zeng, P., & Hou, L. W. (2004). Apparent protective effect of high density lipoprotein against coronary heart disease in the elderly. *Chinese Medical Journal (England)*, 117(4), 511–515.
- McKnight, G. M., Duncan, C. W., Leifert, C., & Golden, M. H. (1999). Dietary nitrate in man: Friend or foe? *British Journal of Nutrition*, 81(5), 349–358.
- Milagro, F. I., Campion, J., & Martinez, J. A. (2006). Weight gain induced by high-fat feeding involves increased liver oxidative stress. *Obesity (Silver Spring)*, 14(7), 1118–1123.
- Miller, M., Seidler, A., Moalemi, A., & Pearson, T. A. (1998). Normal triglyceride levels and coronary artery disease events: The Baltimore coronary observational long-term study. *Journal of the American College of Cardiology*, 31(6), 1252–1257.
- Moore, K., & Roberts, L. J. 2nd, (1998). Measurement of lipid peroxidation. *Free Radical Research*, 28(6), 659–671.
- Navab, M., Hama, S. Y., Anantharamaiah, G. M., Hassan, K., Hough, G. P., Watson, A. D., et al. (2000). Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: Steps 2 and 3. *Journal of Lipid Research*, 41(9), 1495–1508.
- Navab, M., Hama, S. Y., Cooke, C. J., Anantharamaiah, G. M., Chaddha, M., Jin, L., et al. (2000). Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: Step 1. *Journal of Lipid Research*, 41(9), 1481–1494.
- Oba, S., Shimizu, N., Nagata, C., Shimizu, H., Kametani, M., Takeyama, N., et al. (2006). The relationship between the consumption of meat, fat, and coffee and the risk of colon cancer: A prospective study in Japan. *Cancer Letter*, 244(2), 260–267.
- Phillips, M. C., Gillotte, K. L., Haynes, M. P., Johnson, W. J., Lund-Katz, S., & Rothblat, G. H. (1998). Mechanisms of high density lipoprotein-mediated efflux of cholesterol from cell plasma membranes. *Atherosclerosis*, 137(Suppl.), S13–S17.
- Pogoda, J. M., & Preston-Martin, S. (2001). Maternal cured meat consumption during pregnancy and risk of paediatric brain tumour in offspring: potentially harmful levels of intake. *Public Health Nutrition*, 4(2), 183–189.
- Qader, S. S., Salehi, A., Hakanson, R., Lundquist, I., & Ekelund, M. (2005). Long-term infusion of nutrients (total parenteral nutrition) suppresses circulating ghrelin in food-deprived rats. *Regulatory Peptides*, 131(1–3), 82–88.

- Reeves, P. G. (1997). Components of the AIN-93 diets as improvements in the AIN-76A diet. *Journal of Nutrition*, 127(Suppl. 5), 838S–841S.
- Ross, R. (1993). The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature*, 362(6423), 801–809.
- Sasaki, S., Horacek, M., & Kesteloot, H. (1993). An ecological study of the relationship between dietary fat intake and breast cancer mortality. *Preventive Medicine*, 22(2), 187–202.
- Shahidi, F., Synowiecki, J., & Sen, N. (1992). Colour characteristics and absence of N-Nitrite-cured seal meat. *Journal of Agricultural and Food Chemistry*, 40(8), 1398–1402.
- Stein, O., Weinstein, D. B., Stein, Y., & Steinberg, D. (1976). Binding, internalization, and degradation of low density lipoprotein by normal human fibroblasts and by fibroblasts from a case of homozygous familial hypercholesterolemia. *Proceedings of the National Academy of Sciences of the USA*, 73(1), 14–18.
- Steinberg, D. (1997). Lewis A. Conner Memorial Lecture. Oxidative modification of LDL and atherogenesis. *Circulation*, 95(4), 1062–1071.
- Steinberg, D. (2002). Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nature Medicine*, 8(11), 1211–1217.
- Steinberg, D. (2005). Hypercholesterolemia and inflammation in atherogenesis: Two sides of the same coin. *Molecular Nutrition in Food Research*, 49(11), 995–998.
- Vasankari, T., Ahotupa, M., Toikka, J., Mikkola, J., Irjala, K., Pasanen, P., et al. (2001). Oxidized LDL and thickness of carotid intima-media are associated with coronary atherosclerosis in middle-aged men: Lower levels of oxidized LDL with statin therapy. *Atherosclerosis*, 155(2), 403–412.
- Walker, B. E., & Kurth, L. A. (1995). Increased reproductive tract tumors in the female offspring of mice fed a high fat diet during the fetal stage of pregnancy. *Cancer Letter*, 97(1), 57–60.
- Willett, W. C. (2000). Diet and cancer. *Oncologist*, 5(5), 393–404.
- Yan, M. X., Li, Y. Q., Meng, M., Ren, H. B., & Kou, Y. (2006). Long-term high-fat diet induces pancreatic injuries via pancreatic microcirculatory disturbances and oxidative stress in rats with hyperlipidemia. *Biochemical and Biophysical Research Communications*, 347(1), 192–199.
- Zambon, A., Hashimoto, S. I., & Brunzell, J. D. (1993). Analysis of techniques to obtain plasma for measurement of levels of free fatty acids. *Journal of Lipid Research*, 34(6), 1021–1028.